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STUDIES ON ANTIHEMOPHILIC GLOBULIN

BY

MARGARETA BLOMBÄCK

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Studies

on

ANTIHEMOPHILIC GLOBULIN

By

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This dissertation is a discussion and summary of the following five papers:

I. BLOMBÄCK, B. and BLOMBÄCK, M.: Purification of human and bovine fibrinogen. *Arkiv f. kemi, 10:* 415, 1956.

II. NILSSON, I. M., BLOMBÄCK, M. and VON FRANCKEN, I.: On an inherited autosomal hemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time. *Acta Med. Scand., 159:* 35, 1957.

III. NILSSON, I. M., BLOMBÄCK, M., JORPES, E., BLOMBÄCK, B. and JOHANSSON, S.-A.: v. Willebrand's disease and its correction with human plasma fraction I—0. *Acta Med. Scand., 159:* 179, 1957.

IV. BLOMBÄCK, M. and NILSSON, I. M.: Treatment of hemophilia A with human antihemophilic globulin. *Acta Med. Scand., 161:* 00, 1958.

V. BLOMBÄCK, M.: Purification of antihemophilic globulin. I. Some studies on the stability of the antihemophilic globulin activity in fraction I—0 and a method for its partial separation from fibrinogen. *Arkiv f. kemi, 12:* 387, 1958.

Historical Aspects

A. General Review of Hemophilia and Antihemophilic Globulin Deficiency

Already in ancient times, not only the severe and often fatal hemorrhages of patients suffering from classical hemophilia were known, but also something about the hereditary pattern of this disease (Talmud). In 1808, Otto published his paper on the clinical and hereditary features of hemophilia, and some thirty years later Lane (1840) observed the delayed coagulation of the blood in a hemophilic boy, and reported the life-saving effect of a direct homologous blood transfusion. The blood groups were evidently compatible. The delayed coagulation in hemophiliacs was confirmed by Wright in 1893 and by Sahli and Weil in 1905. In addition, they found a normal fibrinogen content, thereby confirming earlier observations, as well as normalization of the coagulation time on addition of normal serum to the hemophilic blood *in vitro* and *in vivo* (Weil). Quantitatively normal prothrombin was found by Morawitz & Lossen (1908) and a normal platelet count by them and by Sahli (1905, 1910).

A series of experiments on hemophilic blood was reported in 1911 by Addis. He found that the formation of thrombin was delayed in hemophilic plasma, and that the coagulation time became normal when a globulin fraction prepared from normal plasma was added to hemophilic plasma *in vitro*, an effect which was not seen when the fraction was prepared from hemophilic plasma. These observations were, however, misinterpreted by the author, who believed that he had prepared prothrombin, and that the prothrombin of hemophilic plasma was an abnormal one. All the same, he clearly pointed out that the globulin fraction was not identical with tissue thrombokinase, since it did not shorten the coagulation time of normal plasma, and that no activity could be noted in serum. In addition, he showed that the clot-promoting principle of the blood cells, observed by Sahli (1910), went into the supernatant if the cells were thoroughly washed.

Feissly (1923) and Frank & Hartmann (1927) suggested that, in hemophilic blood, proserozym is converted to serozym (prothrombin) more slowly than in normal blood. They ascribed the normalizing effect of normal platelets on the coagulation time of hemophilic plasma, observed by Fonio (1915), to normal proserozym adsorbed to the platelets. In 1924, Feissly provided definite evidence of the normal function of the hemophilic platelets. When he administered

platelet-free plasma (prepared in paraffin-coated tubes) to hemophiliacs, he observed an equally good effect on the coagulation time as with usual plasma or normal blood. He wrote as follows:

"... l'injection intraveineuse de sang normal est seule capable de corriger nettement le retard de la coagulation du sang hémophilique. Cette action doit être attribuée au plasma et non point aux éléments cellulaires, car le plasma privé de tous les éléments cellulaires par une centrifugation suffisante, exerce les mêmes effets que le sang total. La transfusion agit en apportant au sang de l'hémophile un 'prosérozyme' normal capable de subir les transformations nécessaires pour acquérir la 'fonction sérozymique', c'est-à-dire de réagir avec le cytozyme, en présence de calcium, pour former une thrombine active."

No effect was seen when plasma from one hemophilic patient was given to another.

Howell & Cekada (1926) found the prothrombin to be normal quantitatively and qualitatively in hemophilia. Frank & Hartmann (1927), as well as Govaerts & Gratia (1931), showed that normal plasma in small concentrations, even if deprived of prothrombin, has a normalizing effect on the coagulation time of hemophilic plasma; plasma so prepared was also used *in vivo* by the former authors. Eagle (1935), Quick (1935) and Brinkhous (1939) likewise found normal prothrombin in hemophilia.

Patek & Stetson (1936) made a thorough investigation of earlier results and showed that there is a factor missing in hemophilia, which is present in normal plasma, but which is not identical with any of the classical coagulation factors. This factor was denoted as antihemophilic globulin (AHG) by Patek & Taylor (1937).

Howell (1939) suggested the existence of some defect in plasma thromboplastin in hemophilia. Delayed transformation of prothrombin to thrombin was shown by *e. g.* Eagle (1935), Brinkhous (1939) and Hecht (1942). Feissly (1945), using the paraffin technique, and Brinkhous (1947) and Quick (1947), using the silicone technique of Jaques *et al.* (1946), were able to demonstrate that platelets (normal or hemophilic) are necessary for the ability of the plasma factor missing in hemophilia to correct the coagulation defect. Thus, the antihemophilic globulin and the platelets normally reacted in some way to generate plasma thromboplastin.

In 1944, Castex, Pavlovsky & Simonetti observed a mutual correction of the coagulation time by the blood of hemophiliacs *in vitro* and *in vivo*, and that in some of their hemophiliacs the transfusion of normal serum was more effective than transfusion of normal plasma. In 1950, Pavlovsky, Mittelman & Castellanos suggested as a possible explanation that the missing factor was not the same in all of their hemophiliacs, and pointed out that one of the factors could be adsorbed by $BaSO_4$. The same suggestion was made by Koller, Krüsi & Luchsinger

(1950), by Schulman & Smith (1952) and by Poole (1953), who all observed a mutual correction by the blood of some of their hemophiliacs. Aggeler *et al.* (1952) and Biggs *et al.* (1952) made thorough investigations of the factor missing in this form of hemophilia, and clearly distinguished it from AHG. Various names were given to it, i. e., PTC (Aggeler *et al.*), Christmas factor (Biggs *et al.*), factor B (Soulier & Larrieu 1953) and factor IX (Koller 1954). Nowadays, hemophilia B and Christmas disease seem to be the names commonly used for this disease.

Different figures are given for the incidence of hemophilia B. In most countries the figure seems to be 10—20 per cent of the hemophilic families, but in Switzerland the figure is probably 50 per cent (*cf.* Soulier 1956, Deutsch 1957).

The observations regarding the factors missing in hemophilia A and B, respectively, had been made possible by critical investigations of the test methods earlier used, and by elaboration of better ones. Among the latter are those of Brinkhous (1939), Quick (1947), Merskey (1950) and Langdell, Graham & Brinkhous (1950), as well as more recent ones, such as the methods of Langdell, Wagner & Brinkhous (1953), Pitney & Dacie (1953) and Soulier & Larrieu (1953), and especially the thromboplastin generation test (TGT) of Biggs & Douglas (1953). It was shown by the experiments of Biggs, Douglas & Macfarlane (1953 a, b) that not only AHG and platelets were necessary for generation of plasma thromboplastin, but also the Christmas factor.

These new methods allowed the detection of mild forms of hemophilia A and B (Graham, McLendon & Brinkhous 1953). Moreover, it was possible to demonstrate that a significantly lower AHG level is present not only in classical hemophilia A, but also in several cases suffering from the inherited hemorrhagic diathesis, with features characteristic of mild forms of classical hemophilia, and associated with a prolonged bleeding time and bleedings from the mucous membranes, the platelet count being normal. From 1953 onwards, several cases of this hemorrhagic syndrome have been found to have a low AHG content in addition to the prolonged bleeding time (Alexander & Goldstein 1953, Larrieu & Soulier 1953, Quick & Hussey 1953, Bigelow 1954, André & Le Bolloc'h-Combrisson 1955, Beller 1955, van Creveld, Jordan, Punt & Veder 1955, Darte 1955, Frischauf 1955, Schulman *et al.* 1955, 1956, Verstraete & Vandenbroucke 1955, Matter *et al.* 1956, Singer & Ramot 1956, Biggs & Macfarlane 1957).

Since this syndrome bears a great clinical resemblance to the disease originally described by von Willebrand (1926), many authors have tried either to distinguish between them or to identify them with each other (*cf.* Biggs & Macfarlane 1957). Deficient prothrombin consumption, indicating a defect in the first stage of coagulation, was found by Jürgens & Forsius (1951) in the patients originally described by von Willebrand. Using the thromboplastin generation

test (TGT), Jürgens, Forsius & Forsell observed a slower generation with the platelets of one of von Willebrand's patients than with normal platelets (cit. Jürgens 1955). This fact was considered as a sign of deficiency of platelet factor 3. In addition, a defect in "agglutination" of the platelets was observed (von Willebrand, Jürgens & Dahlberg 1934, Jürgens & Ferlin 1950, Jürgens, Forsius & Forsell 1955, Dörken & Landbeck 1955).

The prolonged bleeding time in von Willebrand's disease has, however, been ascribed by many workers to a defect in vascular constriction or in the capillary wall (von Willebrand *et al.* 1931, 1934, Macfarlane 1941, Perkins 1946, Lelong & Soulier 1950, Revol, Favre-Gilly & Ollagnier 1950, Macfarlane & Simpkiss 1954) but no defect could be found e. g. by Koch & Schultze (1952), Jamra *et al.* (1952) and Braunsteiner & Pakesch (1956). In the aforementioned patients of Alexander & Goldstein, Schulman *et al.* and Matter *et al.*, a defect in the capillary wall as well as a low AHG content was found. However, since the platelets were found to behave normally in the TGT, these authors pointed out that patients with a clinical diagnosis of von Willebrand's disease must be divided into at least two entities, those in whom there is a deficiency of AHG, and those with a defect of the platelets in the TGT. Soulier & Alagille (1956), as well as Klesper & Achenbach (1957), suggested still more entities.

In the past few years, other hemorrhagic, 'hemophilia'-like syndromes with a deficiency in the plasma thromboplastin generation have been described, such as PTA (Plasma Thromboplastin Antecedent) deficiency (Rosenthal 1954) and Hageman factor deficiency (Ratnoff 1954), as well as combined deficiencies of different kinds.

B. Purification of AHG and Treatment of Hemophilia A

Despite the good hemostatic effect of the homologous blood transfusion given to a hemophilic boy by Lane (1840), reports of shock very probably prevented its use during the second half of the 19th century. After the discovery of the blood groups by Landsteiner in 1901 and the rediscovery by various authors of the delayed coagulation in hemophilia, blood transfusion therapy became more commonly applied.

The good hemostatic effect of this therapy was witnessed by several authors. A review of the literature and a report of the favourable results in his own cases was given by Sköld in 1944.

In the meantime, investigations of different plasma globulin fractions correcting the coagulation time of hemophiliacs both *in vitro* and *in vivo* were reported.

The *in vitro* observation made by Addis in 1911, that a globulin fraction prepared from normal human plasma corrected the coagulation time of hemophilic

blood, was confirmed *in vitro* and *in vivo* by Bendien & van Creveld (1935, 1936), and independently by Patek & Taylor (1936, 1937). The latter authors also made a more thorough investigation of the stability of the antihemophilic activity in their fraction, prepared by dilution and acidification of plasma. They observed *e. g.* that the activity was destroyed by heating at 56° C for five minutes. They found no or an insignificant activity if the fraction was prepared from hemophilic plasma.

A somewhat modified preparation was made by Pohle & Taylor (1937, 1938). However, the fraction also decreased the coagulation time of normal blood. A refractory period was obtained after some injections *in vivo* to the hemophilic patients, although they still responded to normal plasma transfusions. Lozner & Taylor (1939) prepared a fraction by dialyzing plasma, and thus obtained preparations which did not produce any refractory period, but still had an effect on the coagulation time of hemophiliacs *in vivo*. The results were confirmed by van Creveld & Mastenbroek in 1941 who, however, found that all the activity was lost when the preparation was filtered through a Pasteur Chamberland filter. Contrary to the findings of Patek & Stetson (1936), the fractions of Bendien & van Creveld (1935, 1936), mentioned above, could also be prepared from serum. The activity *in vivo* was claimed to be fairly good, but no data were given. A drawback was the temperature elevation always seen. Further purification was performed by means of adsorption on activated charcoal and elution by sodium desoxycholate.

The results of these earlier authors are difficult to evaluate, since the methods used for assay were insensitive (coagulation time) and some of the negative results might have been due to the fact that some of the patients suffered from hemophilia B.

During World War II, two important methods were elaborated for plasma fractionation, i. e., Cohn's method 6 (1946) and the method of Kekwick, Mackay & Record (1946). In both these methods the AHG was precipitated in the fibrinogen fraction (Edsall *et al.* 1944, Taylor *et al.* 1945, Minot *et al.* 1945, Lewis *et al.* 1946, van Creveld & Mastenbroek 1946).

These fractions were also used *in vivo*, but the first enthusiasm over the good effect was counteracted by the observation that, despite the normalized coagulation time, the patients treated during a bleeding episode often continued to bleed. The reason for this failure was first detected by Alexander & Landwehr (1948), who showed that the methods earlier used to measure the AHG activity were not sensitive enough. Many further investigations were made (Beaumont, Caen & Bernard 1954, Brinkhous 1954, Brinkhous *et al.* 1954, Langdell, Wagner & Brinkhous 1955, Brinkhous *et al.* 1956 and Biggs & Macfarlane 1957). Normal coagulation times were observed at AHG levels above 1—5 per cent of normal, and normal prothrombin consumption at AHG levels above 2—10 per

cent of normal. Hörder (1957) also noted a normal TEG (thromboelastogram) above 10 per cent AHG. According to the aforementioned authors, levels of at least 15 to 35 per cent of normal must be maintained to ensure satisfactory hemostasis (Brinkhous *et al.*, Biggs & Macfarlane). About 3 litres of blood would be needed to raise the AHG level to about 35 per cent, and this still does not allow for the rapid consumption of AHG in flask blood *(cf.* Penick & Brinkhous 1956). The AHG consumption is also very rapid *in vivo;* a half-life of 2—3 hours was observed by Langdell, Wagner & Brinkhous (1955) in hemophilic dogs. A half-life of 6—12 hours in hemophilic patients was found by Deutsch (cit. Achenbach) and by Biggs in 1957.

In the past few years, methods for purification of antihemophilic globulin have been devised by several workers. Thus, Bidwell (1955) succeeded, by precipitating with phosphate and citrate, in purifying the AHG from bovine plasma up to 100 to 400 times per mg nitrogen. In comparison with normal human plasma, the activity was about 13 times more per mg nitrogen. In 1957 preparations with still greater activity were reported (Macfarlane *et al.*). Good results have been obtained *in vivo* (*cf.* Macfarlane *et al.* 1957), although the antigenicity of the preparation limits its use (Sharp & Bidwell 1957). Van Creveld *et al.* (1956), starting with the fractions of Cohn *et al.* (1946) and of Kekwick *et al.* (1946), tried to purify the AHG, removing the fibrinogen by precipitation at 56° C. A purification of at least 28 times compared with plasma on a protein basis was obtained, but heavy losses were observed. Some *in vivo* results were described, but are difficult to evaluate. The preparation could be further purified by adsorption on kaolin, but in this step as well the loss of activity was great.

Spaet & Kinsell also made use of the heating procedure. Starting with heated (56° C) bovine Cohn's fraction I, they adsorbed the impurities on $BaSO_4$ and, after precipitation at pH 5.4, a product 70 times more active per mg protein than fresh human plasma was obtained. Shinowara (1956), according to a preliminary report, succeeded in separating the antihemophilic globulin or, as he denotes it, TPC (Thromboplastic Plasma Component) from the fibrinogen in Cohn's fraction I by precipitating the fibrinogen with ether. A good yield of both substances was obtained; the TPC was contaminated with less than 3 per cent of fibrinogen. The coagulability of the fibrinogen in the precipitate was 65 to 80 per cent. The TPC could be further purified by ether precipitation in the presence of glycine. The author stated that 2 ml of a 4 per cent solution was equivalent to 300 ml of citrated plasma when assayed on hemophilic whole blood. No mention of *in vivo* application was made in this report.

Wagner & Thelin (1956) and Wagner, Richardson & Brinkhous (1957) were able to separate the fibrinogen from the AHG by selectively adsorbing the fibrinogen of plasma on fuller's earth. The AHG could be further purified by

salting-out or by ethanol precipitation. A product 200 times more active per mg protein than the starting material (canine plasma) was obtained.

In 1957, Kekwick & Wolf described a modification of the method of Kekwick *et al.* (1946). A preparation was obtained which still contained fibrinogen, but which had an AHG activity of 20—25 times that of human plasma per mg protein. The effect *in vivo* was as good as *in vitro*.

In 1957, Surgenor & Steele reported good separation of the AHG activity from the fibrinogen in a modified fraction I. This was achieved by precipitating the fibrinogen with dextran sulphate of high molecular weight, leaving the AHG in the supernatant. It could be further purified by dialysis. No mention was made of its effect *in vivo*. However, the fibrinogen could not be recovered after the treatment with dextran sulphate.

In view of the extensive literature on the subject, this survey has been confined mainly to the publications relevant to the present investigations.

Present Investigation

Introduction

During some purification work on the heparin co-factor in 1953, it became evident that no reliable assay could be performed without a stable fibrinogen. The fibrinogen preparations used, which had been prepared according to a number of different methods, were, however, instable, mainly due to activation of contaminating prothrombin to thrombin in the fibrinogen solutions.

Work was therefore started with the object of purifying fibrinogen and obtaining a stable preparation.

It proved possible to prepare a stable fibrinogen in good yield from Cohn's fraction I, by introducing glycine in order to "salt out" the fibrinogen, and at the same time "salt in" the impurities into an aqueous glycine-ethanol-citrate mixture (Paper I). The fraction thus purified was denoted as fraction I—0.

By making use of variations in ionic strength, ethanol and glycine concentrations, fraction I—0 could be separated into fraction I—1, containing the bulk of cold-insoluble globulins, and fraction I—2, containing a further purified fibrinogen. Fraction I—2 could, in turn, be separated into fraction I—3, containing impurities, and fraction I—4, consisting of a practically pure fibrinogen.

Since Cohn's fraction I was known to contain antihemophilic globulin (AHG) activity, it was considered of interest to ascertain whether the purified fibrinogen fractions were "contaminated" with AHG. Together with I. M. Nilsson, the different fractions were assayed for AHG activity. Practically no activity was found in the fibrinogen fractions I—2 and I—4, nor in fractions I—1 and I—3, but in fraction I—0, bovine or human, the yield when tested *in vitro* was good (Paper I). At that time, Nilsson had observed a female patient (Case 1, Paper II) with an AHG content of < 5 per cent of normal and prolonged bleeding time, who required a large number of blood transfusions at each menstruation. It was therefore decided that, if fraction I—0 could be prepared in a sterile, pyrogen-free form, retaining its AHG activity, this fraction would be given to the patient in question, to determine whether it could temporarily normalize her coagulation defect.

Both the aseptic closed-system technique of Kekwick & Mackay (1954), some-

what modified, and sterile filtration were initially used (cf. Paper I). The activity of human fraction I—0 before sterile filtration was almost quantitative in comparison with fraction I. However, the loss in filtration was great.

In May 1956, severe reactions to whole blood (high fever and urticaria) had developed in the aforementioned patient. A preparation of fraction I—0 was administered to her by Nilsson (Nilsson 1957, Nilsson, Blombäck, Blombäck & Svennerud 1956). The AHG content rose to the expected level and, unexpectedly, the bleeding time became normal. Hysterectomy was then performed immediately under cover of new doses of fraction I—0. Somewhat later another patient, a boy with classical hemophilia A (Case 1, Paper IV), was treated with fraction I—0 with equally good results.

In the meantime, Nilsson started an investigation of the family of the first patient. She found not only a low AHG content in the mother, but also a distant relative i. e., the maternal great-great-grandmother's sister's granddaughter, with a severe form of the same disease. Additional patients with the same hemorrhagic diathesis were traced. An investigation of the content of the various coagulation factors, especially the AHG, in the members of the families of these patients was made by Nilsson, von Francken and the present author (Paper II). The work was continued by an investigation (Paper III) of the patients on the islands of Åland, originally described by von Willebrand, with clinical features greatly resembling those in our patients. A low AHG content was demonstrated in the Åland patients as well.

Fraction I—0 was administered both to some of these patients (Papers II and III) and to 12 patients with hemophilia A in connexion with acute bleedings, such as gastrointestinal bleedings, and joint bleedings, as well as in connexion with operations, for instance, appendectomy and tooth extractions (Paper IV). During this work it was, however, observed that the yield of AHG in fraction I—0 was not consistently good. Various conditions were therefore changed to improve the yield (Paper V).

In the early stages of the present investigation, attempts were made to separate the fibrinogen from the AHG in fraction I—0. A fairly good yield of both substances could be achieved by precipitating the AHG activity from solution at low ionic strength in the presence of glycine and a low ethanol concentration. Most of the fibrinogen remained in the supernatant and could be recovered from it.

The primary problem, however, soon became that of the poor yields often obtained in fraction I—0. The work on the separation of AHG from fibrinogen was therefore postponed and has only recently been resumed (*cf.* Paper V).

In the following, a summary will be given of the aforementioned investigations, and some new aspects of the activities found in fraction I—0 will be presented. Some of the relevant literature will also be reviewed.

Experimental

A. Methods of Assay

A detailed description of the methods has been given in Paper II.

1. Assay of AHG

Storage of the samples. — As can be concluded from Paper V, the samples of AHG preparations to be tested had to be frozen rapidly and stored at — 23° C or below, if not tested immediately. The plasma samples of control and patient, as well as hemophilic A plasma, were also stored at this temperature.

The AHG activity in the plasma of the patients was assayed:

(a) by Biggs, Eveling & Richards' (1955) modification of the thromboplastin generation test; the oxalated plasma was adsorbed with $BaSO_4$ (*cf.* Verstraete & Vandenbroucke 1956).

(b) on hemophilic A plasma (with less than 1 per cent of AHG) in a recalcification system, in which the ability of the control and the test plasma to correct the prolonged recalcification time of the hemophilic plasma is compared. The plasma of the patients was tested in dilutions 1/10, 1/20, 1/50 and 1/100 (in saline), and that of the control in 1/10, 1/20, 1/50, 1/100, 1/200 and 1/500. Citrated plasma was used in this method.

Standard for the assay of AHG and other factors in the patients. — Use was made of plasma from a control subject (I.M.N. or M.B.) having, in addition to a normal content of the other clotting factors, an AHG content which coincided with the mean AHG content in the plasma of 20 healthy subjects, in whom the range was found to be 60—160 per cent, if 100 per cent is defined as the mean value. The standard deviation was found to be 17.5 per cent of the normal value, 100 per cent (Paper II).

It can be recalled that the samples were taken with every precaution, using the silicone technique. It can also be mentioned that no blood samples for AHG assays were taken during infection or menstruation. In patients with the slightest suspicion of AHG deficiency, two samples were invariably taken. Only samples in which venipuncture had been performed without any complications were used for the assays; the importance of this detail has also been stressed by Tocantins (1955) and Penick & Brinkhous (1956), among others. It was checked that the hemophilic plasma used as a test basis contained the normal amounts of other known coagulation factors, and that no anticoagulant was present (Lewis, Ferguson & Arends 1956).

The AHG activity in the various fractions was assayed by the method using hemophilic A plasma (see above). Dilutions of 1/50 to 1/800 in saline were, for

instance, used for the AHG preparations. The supernatants, however, could often not be diluted more than 1/10, since the activity per ml was very low.

Eventual thromboplastin activity of the fractions was tested by adding the fraction diluted 1/10 to hemophilic B or normal plasma, and determining whether it shortened the recalcification time.

Standard for the assay of preparations. — Initially, the purification was followed by comparing the activity of the various fractions with that of the starting material, i. e., the original plasma. Subsequently, use was made instead of freeze-dried human fraction I—0, reconstituted in distilled water and containing 3.5 times the AHG activity of normal plasma per ml. The same fraction I—0 has been used throughout. It was assayed in 4 or 5 dilutions (1/50 to 1/800).

Because of the shortage of hemophilic plasma, attempts were made to circumvent its use; for example, various artificial test bases were tried. Thus, purified fibrinogen was added to the supernatant of Cohn's fraction I. The supernatant had been freeze-dried and dissolved before use in distilled water to plasma volume (*cf.* Wolf 1956). This was not, however, a stable substrate even if prepared from hemophilic plasma; the recalcification time of the blank was reduced after a short interval, probably indicating activation of the prothrombin in the mixture. Dissolving the freeze-dried supernatant in various buffers gave no better results. Several further modifications were tested without success.

Hemophilic plasma was therefore used throughout the investigation. The activity of the preparations and the results of the treatment of the patients in Papers II, III and IV were followed only with this method. The chief advantage of the method is that a more adequate test base than hemophilic A plasma can scarcely be envisaged, since all the coagulation factors with the exception of the AHG are present in normal quantities. However, it is necessary, even with this method, to test whether the activity of the preparation may not be due to thromboplastin activity. Other authors have investigated the effect of blood, plasma or AHG fractions on the AHG level in hemophilic patients with various methods e. g. those of Geiger, Duckert & Koller (1956), Pitney (1956), Wolf (1956) and Biggs (1957).

Another fact emphasizing the advantages of this method is that it was possible, with its use, to demonstrate a low AHG content (20 to 60 per cent of normal) in the plasma of 23 definite carriers of hemophilia A before the menopause (Nilsson *et al.;* to be published) (*cf.* Verstraete & Vandenbroucke 1955).

A serious drawback is the difficulty of obtaining plasma from hemophiliacs in whom no anticoagulant has developed, and with an AHG content of less than 1 per cent. Moreover, the method is somewhat time-consuming, even when run by two persons, and the procedure should, in view of the relative instability of hemophilic plasma, be completed within two and a half hours at the most.

2. Assay of the factor correcting the bleeding time

As far as we are aware, this factor can only be tested *in vivo* in patients in whom it is lacking. Consequently, a quantitative determination of this activity cannot at present be made. It can be roughly estimated from the clinical results, i. e., the degree of correction of the prolonged bleeding time and the duration of the effect. The bleeding time in the patients was determined with the method of Duke, using standardized hemolets (Dade Reagents, Inc.). Determinations were usually made in both ears.

3. Methods for the assay of other coagulation factors

The other methods used are described in Paper II. It should be mentioned that the values for prothrombin consumption are somewhat unreliable, since the interval elapsing between collection of the blood (patient and control) and withdrawal of the serum varied considerably.

4. Method for determination of total protein and fibrinogen

The method is outlined in Paper I and described in detail by B. Blombäck (1958).

Total protein in the preparations. The sample is diluted to a protein concentration of about 0.15 to 0.35 per cent. Alkaline urea is added and the extincion read spectrophotometrically at 282 mμ.

Fibrinogen in the preparations or in the plasma samples was determined as fibrin, after coagulation of the sample with bovine thrombin at pH 6.3—6.4, ionic strength about 0.15 and a fibrinogen concentration of 0.05—0.12 per cent in the coagulation mixture. The fibrin formed was collected by syneresis and dissolved in alkaline urea for the subsequent spectrophotometric analysis.

The *coagulability* of the preparations was determined as the ratio of coagulable protein to total protein. In calculating the coagulability, the extinction coefficient ($E\ \frac{1\ \%}{1\ cm}$ at 282 mμ) of fibrinogen was used for the coagulated sample as well.

B. Fraction I-0

1. Preparation (Paper I)

Since proteins as a rule are more stable in the solid state, attempts were made to purify the fibrinogen in Cohn's fraction I by extracting the impurities, including prothrombin, of this fraction under conditions where activation of the latter was not likely to occur, and where the fibrinogen was practically insoluble. The advantages of extraction procedures have been pointed out by Cohn *et al.* (1950). Moreover, extraction is more easily done under sterile conditions than is precipitation.

Preliminary experiments showed that dissociation of the protein complexes

and separation of the components of fraction I were possible when this fraction was extracted in the presence of glycine. Glycine in high concentrations enhanced the salting-out effect on the fibrinogen, i. e., reduced its solubility, whereas the salting-in effect on the contaminating proteins was large, i. e., most of them went into solution.

The most suitable extraction solution for removal of the impurities from human fraction I, leaving the fibrinogen in the residue, was found to be: 0.055 *M* citrate buffer, pH 6.0 (*cf.* point 2), 1 *M* glycine, containing 6.5 per cent (v/v) of ethanol. If human fraction I was extracted twice at — 3° C with this mixture, 95 per cent of the fibrinogen was left in the residue, whereas the main part of the impurities were removed in the supernatants. The coagulability of the fibrinogen fraction, denoted as I—0, was 85 to 90 per cent.

Only traces of the prothrombin activity of the original plasma were found in this fraction. Active plasmin has never been found, but traces of plasminogen and its proactivator were still present. When fraction I—0 was tested for its eventual AHG activity, it was found to remain to a large extent in this fraction.

The fibrinogen in this fraction was further purified by precipitating cold-insoluble globulins and other impurities at high ionic strength (0.3) in the presence of glycine (0.12 *M*) and a low concentration of ethanol (2.0 per cent) (fraction I—1). By raising the ethanol concentration to 6.5 per cent, the fibrinogen (I—2) could then be precipitated from the supernatant of fraction I—1 in a yield of 65—75 per cent of fraction I, with a coagulability of 94—97 per cent for human fibrinogen. If a still purer fibrinogen was desired, further impurities could be removed from the dissolved fraction I—2 by precipitating a fraction (I—3) at low ionic strength (0.1) in the presence of glycine (0.5 *M*) and ethanol (0.75 per cent). By raising the ethanol concentration to 6.5 per cent, the fibrinogen (I—4) was recovered from the supernatant in a yield of 60—70 per cent of that in fraction I—2. It was coagulable to 98—100 per cent with thrombin. The method was applied to bovine material with equally good results.

2. Yield and stability of AHG in fraction I—0 (Paper V)

As stated in Papers I and II, the yield of AHG in human fraction I—0 was good. However, on sterile filtration through bacteriological glass filters, heavy losses were observed, sometimes up to 75 per cent of that before filtration (*cf.* Paper I). No improvement resulted from the use of other types of filter. As it was found that the preparations were sterile even before filtration, this step was omitted and the fractions were prepared exclusively in an aseptic closed system. During the course of the work, fraction I—0 was for some unexplained reason occasionally obtained with a poor yield of AHG. Some of the

preparations were more active *in vivo* than *in vitro*. This might have been due to the fact that the samples taken for assay had been slowly frozen at —10° C to —25° C, in contrast to the bulk of the preparations, which were shell-frozen at —30° C to —50° C. But some preparations had a low activity both *in vivo* and *in vitro*. The conditions for preparation of fraction I—0 were then varied in different ways, *e. g.* by a slight increase in the amount of citrate used as the anticoagulant, and by introducing magnetic stirring during collection of the blood. Although the latter procedure resulted in an improvement, the yields were not invariably good (Papers IV and V).

Since it was believed that the optimal conditions as regards glycine concentration and pH of the extraction solution for obtaining a good yield in fraction I—0 might not be the same for the AHG activity as those for fibrinogen, this matter was investigated. The experiments were performed on a small scale. As far as glycine was concerned, it was found that variations in concentration led to no better results.

As to pH, Bidwell (1955) reported the optimal pH for the stability of bovine AHG activity to be 6.8 (*cf.* Wagner & Thelin 1957). It was therefore thought that a better yield of AHG might be obtained if the extractions were performed at a somewhat higher pH than 6.0, i. e., that at which human fraction I had earlier been extracted. Extractions were therefore performed at different pH values, i. e., 6.0, 6.3, 6.8 and 7.3. As can be inferred from Paper V, not only was the solubility of AHG less in the extraction solution at pH 6.8, but the stability was also greater at this pH. The preparative technique was therefore changed accordingly. Both the yield of fibrinogen and the coagulability in the modified fraction I—0 seem to be the same as those obtained with the original technique (Paper V).

In the beginning of the present investigation, the yield of the AHG activity in fraction I—0 was found to be almost quantitatively that of fraction I. The purification in fraction I—0 was 30 to 50 times that of the original plasma in terms of the activity per mg protein (Papers I and II). Later, when lower yields were obtained, the purification in some batches was as low as 10 times or even less (Paper IV). When the pH of the extraction mixture was recently changed to 6.8, good purification was achieved.

3. Partial separation of the fibrinogen from the AHG in fraction I-0 (Paper V)

When the fibrinogen in fraction I—0 was further purified (Paper I), the AHG activity disappeared. In this procedure, some of the cold-insoluble globulins are precipitated at high ionic strength (0.3) in the presence of glycine in low concentration (0.12 *M*), whereas the rest of the cold-insoluble globulins are precipitated at lower ionic strength (0.1) in the presence of glycine in a some-

what higher concentration. When this last step was applied directly on bovine fraction I—0, it was found that the AHG activity was recovered in the precipitate, although still contaminated with fibrinogen. The bulk of fibrinogen could then be precipitated from the supernatant by raising the ethanol concentration.

As described in Paper V, it was found that the AHG activity could best be separated from the fibrinogen in human fraction I—0 in citrate solution (pH 6.8) by precipitating it at low ionic strength (0.1) in the presence of glycine (0.3 *M*) and a low concentration of ethanol (0.5 per cent). The fibrinogen remaining in the supernatant could be recovered from it by raising the ethanol concentration to 6.5 per cent. The yield of AHG in the first precipitate, denoted as I-1-A, was about 80 per cent of that in fraction I—0, and the yield of fibrinogen in the second precipitate, I-2-F, was 70 per cent. The fibrinogen fraction was coagulable to 92 per cent with thrombin, and the AHG fraction to 70 per cent. The purification of the AHG, in terms of activity per mg protein, was 4.5 times that of fraction I—0 and about 100 times that of normal plasma.

Glycine in the concentrations used by the author was originally introduced to increase the solubility of fibrinogen, and the ionic strength was lowered to increase this effect. Whatever the importance of glycine may be, no good separation has hitherto been obtained in its absence.

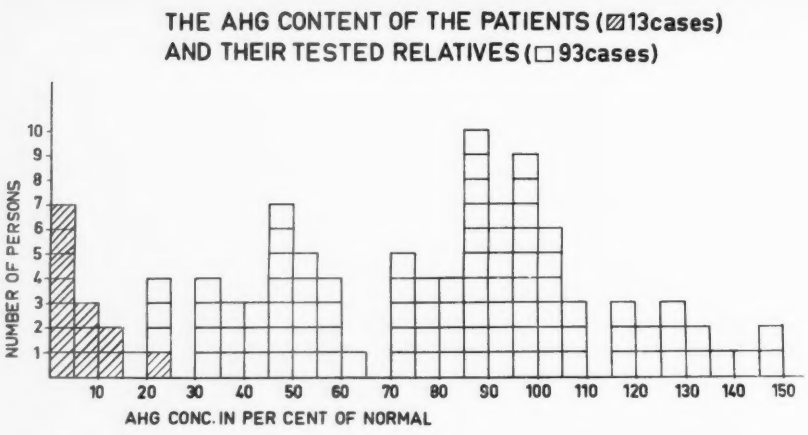
Clinical Investigations

These investigations were performed in collaboration with Dr. Inga Marie Nilsson, Allmänna Sjukhuset, Malmö.

Paper II. *On an Inherited Hemorrhagic Diathesis with Antihemophilic Globulin (AHG) Deficiency and Prolonged Bleeding Time.* As mentioned in the introduction, not only Nilsson's female patient, but also eight other patients (3 males and 5 females) suffering from the hemorrhagic syndrome, with the clinical features of mild hemophilia, as well as bleedings from the mucous membranes, were found to have a low AHG content (2—10 per cent of normal in 6 cases and 10—20 per cent in the remaining 3) in addition to the prolonged bleeding time.

No capillary abnormalities were found at ophthalmologic examination in two cases. The platelets were normal, as was the platelet function with respect to platelet factors 1, 3 and 4.

An investigation of the coagulation factors in 93 of the patients' relatives showed the following. Only one of the parents in each family had a significantly low AHG content (less than 65 per cent of normal). Low AHG values were found in several of the siblings of the parent in question, equally distributed between males and females. Subjects low in AHG had children with both normal and abnormal AHG values. In the affected persons, the AHG values ranged from 2 to 62 per cent of normal (Fig. 1).



The gene causing the AHG deficiency was considered to be autosomal, dominant and with varying expressivity.

Paper III. *v. Willebrand's Disease.* In the patients described above, the platelets, carefully washed before testing, were found to behave normally in the TGT (thromboplastin generation test) of Biggs & Douglas. On the other hand, Jürgens, Forsius & Forsell (cit. Jürgens 1955) had found an abnormal behaviour of the platelets in the von Willebrand patients, suggesting a deficiency of platelet factor 3, as well as a normal content of AHG and Christmas factor in the TGT. Consequently, it was believed at the time that these hemorrhagic disorders were two different syndromes (Paper II). However, a patient in Stockholm, born on Åland (with an ancestor who had presumably suffered from von Willebrand's disease), who had had bleeding symptoms and one of whose sons had bled to death at 7 days of age, was investigated. It was found that she had a low AHG content (50 per cent) and that her platelets behaved normally in the TGT. This indicated that von Willebrand's disease was similar to or identical with the bleeding syndrome described above.

In order to obtain more definite confirmation of these observations, the investigation was continued on the islands of Åland. Including the patient mentioned above, 15 patients with bleeding symptoms were investigated; most of them belonged to the families studied by von Willebrand *et al.* (1926, 1931, 1934). These patients were not, however, severely affected. The same results as above were found, i e., a low AHG content (25 to 60 per cent of normal) and a normal function of the platelets in the TGT as well. The effect of fraction I—0 was tested in a more severely affected patient, and was found to normalize both the coagulation defect and the bleeding time. The patients were kindly placed at our disposal by Professor Jürgens and his Finnish co-workers.

Further investigations disclosed additional patients in Sweden with AHG deficiency and prolonged bleeding time. At the time of writing, altogether 18 patients (8 males and 10 females) with severe clinical bleeding symptoms have been traced in totally 14 families. These patients have a markedly prolonged bleeding time, whereas in the family members a normal value was recorded for the bleeding time, even with AHG values as low as 20 per cent of normal.

At about the same time as Papers II and III appeared, several reports on this subject were published by other workers, *e. g.* that of Jürgens, Lehmann, Wegelius, Eriksson & Hiepler on their investigations on Åland. In 7 of 14 patients investigated, they found a decreased AHG content, 10—70 per cent of normal. We had found a low AHG value in 15 out of 16 subjects investigated. If, however, the patients (O. S., A. S., R. S., A. E., BoE., H. B.) investigated by both groups are compared, it is seen that both teams found a normal AHG content in O. S., the father of a family with many "bleeders". A low AHG content was found by both teams in the other five "common" cases. However, in contrast to our findings, Jürgens *et al.* still found a delayed thromboplastin generation test (*cf.* Jürgens 1955) when the normal platelets were replaced by those of the patients.

At the same time, Achenbach and Achenbach & Klesper and Klesper & Achenbach (1957) reported their investigations of some families with a similar hemorrhagic diathesis; they also noted a low AHG content but no defect in the platelets in the TGT. In addition, they reported on a family with deficiency of factor B or the Christmas factor and prolonged bleeding time. Deutsch (1957) described a family (a man and his mother) with a combined deficiency of factor V, AHG and platelet factor 3. Papers on this subject were also published in 1957 by Gross, Mammen & Illig and by Brockhaus, as well as by Koch *et al.*

Therapy with Fraction I-0

In fibrinogen deficiencies

Fibrinogen has been prepared on a large scale from outdated blood, in principle according to the glycine method (Kabi AB), and has been used with good results in fibrinogen deficiencies.

In the inherited hemorrhagic diathesis described above

The therapy of this disease, or of syndromes with the same clinical features, has been discussed by several workers. The first to use blood transfusions was Minot (1928). Under this cover, an operation was performed. However, since the bleeding time was prolonged already on the next day, he pointed out that the operation could probably have been performed without this measure. Blood transfusion therapy was suggested by von Willebrand in 1931 for the patients

described by him. In 1955, Jürgens reviewed the therapy hitherto used in these patients, and recommended local application of thrombin, as well as blood or plasma transfusions, for the parenchymatous bleedings. Alexander & Goldstein (1953) and Singer & Ramot (1956) observed no effect on the bleeding time after blood transfusions. In one patient, Schulman *et al.* (1956) noted a normalizing effect of a plasma transfusion on the bleeding time.

As already stated, it was found possible with the glycine method to prepare a sterile fraction I—0, with a high AHG content, from fresh human plasma. Since severe blood transfusion reactions had developed in the first patient observed in Sweden with the aforementioned hemorrhagic disorder, she was treated with fraction I—0 (May 1956). Not only did the plasma AHG rise after the injection but, unexpectedly, the prolonged bleeding time was normalized, thus indicating that the latter was to be attributed to the absence of some factor normally present in the plasma. Hysterectomy was performed under cover of further doses of fraction I—0. No blood or plasma transfusions were given.

The first report on this subject was given by I. M. Nilsson (Nebenwirkungen von Arzneimitteln auf Blut und Knochenmark, Internationales Symposion, Malmö, Schweden, Juni 1956. Ed. R. Jürgens und J. Waldenström, Schattauer Verlag, Stuttgart, 1957).

This girl was once more treated in October 1957, when some teeth had to be extracted. The response of the patient's plasma AHG to the fraction was still as good as on the first occasion. She was given the fraction altogether 13 times.

Fraction I—0 was administered to another seven patients of this kind. The effect on the coagulation defect, i. e., on the AHG level of the patient's plasma, as well as on the bleeding time was as satisfactory as in the first patient.

Three of these additional patients are described in Paper II (Cases 5, 6 and 9). Case 6 was treated in connexion with a tooth extraction and Case 9 because of a large hematoma. Two patients have been treated because of intra-abdominal bleedings, one (K. P.) because of epistaxis, and two patients (one of them Case 5) to ensure that their response to fraction I—0 was satisfactory. As mentioned earlier, the same good results were obtained in the patient on Åland (Paper III).

The effect on the bleeding time was as good irrespective of whether the fraction had been prepared from plasma with a high or low platelet content.

Since the normalizing effect on the bleeding time could in all probability be attributed to a plasma factor not hitherto described, *fraction I—0 without AHG activity* was administered to a patient with this disorder. The fraction in question was a sterile-filtered fraction I—0, in which all AHG activity had been removed by filtration. It can be inferred from the results in Cases 5 and 6 (Paper II) that it still had a normalizing effect on the bleeding time, as well as on the bleedings from the gums. As could be expected, the coagulation time was

not normalized, nor did the AHG content increase. The same results were observed in two other patients (Cases M. A., Fig. 2, and K. P.).

Fibrinogen from outdated blood was administered to two patients, in order to ascertain whether purified fibrinogen in itself had any effect on this bleeding disorder. No effect was observed either on the bleeding time, or on the patients' AHG content (*cf.* Fig. 2).

One patient was given not only the usual fraction I—0, but also that without AHG activity, as well as fibrinogen from outdated blood. The effect was that already described. Cortisone, ACTH, Premarine (conjugated estrogens) and progesterone were also administered (Fig. 2). No normalization of the defects was seen.

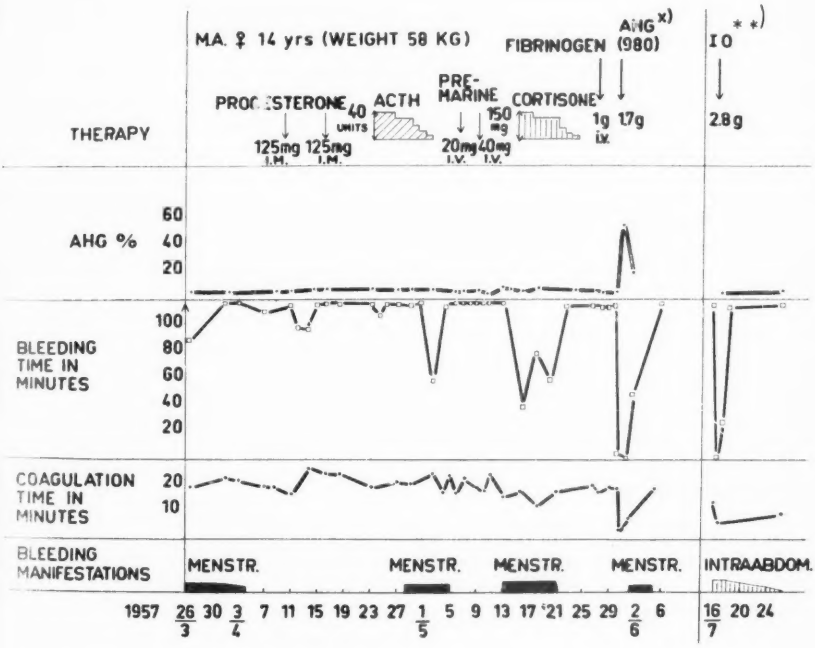


Fig. 2. Effect of different therapy in Case M. A.
*) Fraction I—0 with AHG activity equivalent to 980 ml of normal blood.
**) Fraction I—0 without AHG activity.

Fraction I—0 from hemophilic blood was given to a female patient (K. P., aged 7 years) with this hemorrhagic disorder, who responded satisfactorily to the usual preparation of fraction I—0. Hemophilic fraction I—0 was prepared from 1,500 ml of plasma from 8 hemophiliacs having an AHG content ranging

from < 1 to 2 per cent. In the dissolved fraction, the AHG activity per ml was only 2 per cent of that of normal human plasma. A total dose of 200 ml was given. The oozing epistaxis ceased. A blood sample was taken two hours after the injection. The coagulation time was as long as before (20 minutes). The AHG level had risen from 6 to 12 per cent of normal. The effect on the bleeding time of the hemophilic fraction I—0 was not only marked, but also of longer duration than that of the usual fraction I—0.

A report on Case K. P. has been submitted for publication in Acta Med. Scand.

A comparison between the disappearance of AHG from the plasma of the patients with this type of hemorrhagic diathesis (Papers II and III) and from the plasma of those with classical hemophilia A (Paper IV) shows the half-life of AHG to be longer in the former type. In some cases it has been observed that the AHG content was higher some hours or even days after the administration of fraction I—0 than immediately after it (*cf.* Cases 6 and 9, Paper II). The reason for this discrepancy is not known, but is now being investigated.

As far as cases of AHG deficiency with prolonged bleeding time are concerned, it is evident that the factor correcting the bleeding time is present not only in normal blood, but in hemophilia A blood as well.

Fresh blood transfusions were administered to some patients. In Cases 6 and 9 (Paper II), they had no effect on the bleeding time, but a slight rise in the AHG content of the plasma could be noted. In some cases, two or more transfusions of whole blood had a certain but not fully normalizing effect on the bleeding time.

In thrombocytopenia and macroglobulinemia Waldenström

Since Cohn's fraction I, devoid of AHG after sterile filtration, had been observed by Cazal *et al.* (1956) to have a good effect in some cases of thrombocytopenia, it was considered of interest to determine whether fraction I—0 contained any factor effective in this disease. Fraction I—0 is prepared from blood taken with the silicone technique, and the plasma is siphoned off after the first centrifugation, and once more centrifuged; consequently, it probably cannot contain any appreciable quantity of platelets.

A preparation from 1,400 ml of plasma, with an AHG activity equivalent to that of 1,000 ml of fresh plasma, was administered to a 61-year-old female patient (weight 89 kg) with thrombocytopenia (14,000 platelets per cu. mm) and severe bleedings. No platelet agglutinins were found in the patient's serum. The AHG content of the plasma increased from 148 per cent to 179 per cent of normal, but no effect was seen on the bleeding time (measured in both ears), which was more than 60 minutes both before and after administration of fraction I—0.

In addition, fraction I—0 was given to a female patient, aged 40, with macro-

globulinemia Waldenström, with retinal hemorrhages and bleedings from the gums. The bleeding time was more than 60 minutes both before and after administration, and the bleedings were not arrested.

In **classical hemophilia A**

Since Cohn's fraction I is known to contain AHG, this fraction has been administered by other workers to hemophilic patients, although the results were often disappointing (*cf.* Papers IV and V). Freshly prepared fraction I has, however, often proved to be more effective (Winterstein 1955, Egli & Kesseler 1956, Hörder 1957).

The difficulties have been heavy losses of AHG, due not only to the instability of the fraction, but also to the different sterile filtration procedures used.

As mentioned earlier, some of the failures might have been due to the fact that certain patients had hemophilia B. In Sweden, 12 of 70 hemophilic families investigated were found to belong to the hemophilia B type, i. e., 17 per cent (I. M. Nilsson, personal communication).

Since the effect of fraction I—0 on the AHG level had been so clearly established in the female patient with AHG deficiency and prolonged bleeding time (Case 1, Paper II), it was also given to patients with hemophilia A.

Twelve patients with hemophilia A have been treated for acute bleedings, such as gastrointestinal bleedings, hematuria, bleedings after tooth extractions and joint bleedings, as well as in connexion with operations, such as appendectomy and tooth extractions.

As a rule, a whole dose or a half dose of fraction I—0 was given; a dose was usually prepared from 1,400 ml of fresh normal human plasma, and contained approximately 2.7 g of fibrinogen. If the yield of AHG was good, the patient's plasma AHG could be raised to 35—50 per cent of normal. By increasing the AHG level to 40—80 per cent of normal and by maintaining it at 20—30 per cent of normal the necessary surgical operations could be performed and the healing completed without abnormal bleeding.

When any local or general infection was present, the AHG consumption was observed to be more rapid, and necessitated fresh doses at closer intervals.

One patient (Case 1, Paper IV), with outstanding, severe bleeding tendency, has been given fraction I—0 33 times in the course of 21 months in connexion with joint bleedings. At the time of writing, the rise in the patient's plasma AHG is still that to be expected from the *in vitro* tests, i. e., no resistance has developed.

Another patient had become sensitized to blood (anti-c); a crush kidney syndrome appeared in connexion with blood transfusions given after extraction of several teeth. Administration of fraction I—0 did not cause any side-reactions and controlled the bleeding from the extraction wounds.

No toxic reactions have been observed in any of the patients to whom fraction I—0 was administered.

Some patients have been given fresh normal blood in order to increase their low hemoglobin level. Only a slight increase in the plasma AHG level was seen (about 5 per cent) when 400—500 ml of citrated blood was given. Thus, it is often difficult to raise the AHG level sufficiently by blood transfusions alone in connexion with an acute bleeding.

These observations are in agreement with those of earlier authors, and illustrate the difficulty often encountered in compensating the AHG deficiency by blood transfusions alone. They also stress the necessity of having active, non-toxic, concentrated AHG preparations for effective treatment of severe hemorrhages in hemophiliacs.

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BY

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ON THE CEREBROSPINAL FLUID IN NORMAL CHILDREN AND IN PATIENTS WITH ACUTE ABACTERIAL MENINGO-ENCEPHALITIS

BY

STEN WIDELL

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INTRODUCTION

Our knowledge of the protein content of the CSF in childhood is still incomplete, owing mainly to imperfections in methods of determination hitherto available. However, the method described by Izikowitz (1941) permits more exact calculation of the amount of protein in the CSF. By means of electrophoresis is has now also become possible to separate the various protein fractions of the CSF and to determine their quantitative inter-relationship. This new method of examination has proved useful in the investigation of certain pathologic conditions of the central nervous system, *e.g.* neurosyphilis and multiple sclerosis.

The purpose of the present investigation was to form a clearer opinion of the amount and composition of the CSF protein in normal children of different ages. Studies were also made on the CSF from children and adults with acute abacterial meningo-encephalitis in different stages of the disease. Particular attention was directed to the connection between the clinical symptoms and the changes in the CSF protein. The cell content of the CSF was also studied systematically.

DEFINITIONS AND ABBREVIATIONS

Children are to be understood as individuals below 15 years of age.
Infants are to be understood as individuals below 1 year of age.
Neonatal period is to be understood as the first 2 weeks of life.

CSF=cerebrospinal fluid.
Cell-count=number of white cells/cmm in CSF.
n=number of cases.
m=mean value.
s=standard deviation.
e=mean error of the mean value.
e_D=mean error of the difference.

On comparison between two groups with standard deviations of roughly the same magnitude ($s_1 < 2 \times s_2$) the pooled standard deviation (S) was calculated, and the mean error of the difference was derived by the following formula:

$$e_D = S \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

When the standard deviations were not roughly equal ($s_1 > 2 \times s_2$), the mean error for each group was calculated, and the mean error of the difference was derived by the following formula:

$$e_D = \sqrt{e_1^2 + e_2^2}$$

Unless otherwise stated, the former formula was used.

Differences exeeding $2.46 \times e_D$ (degrees of freedom ≥ 30) were considered of statistical significance (corresponding to a probability of identity of < 0.02).

Chapter I

METHODS

DETERMINATION OF TOTAL PROTEIN CONTENT OF CSF

The total protein content of the CSF was determined by the method of IZIKOWITZ (1941) as modified in 1943.

Principle

The total protein is precipitated with trichloroacetic acid, repeatedly centrifuged and washed to remove all nonprotein nitrogen, and finally combusted at 290° C in the presence of sulphuric acid. The nitrogen content is then determined iodometrically according to TEORELL's (1928) micro-method, slightly modified.

Analytic procedure

The procedure adopted was that described in 1943 by IZIKOWITZ with the exception that the samples were centrifuged and trichloroacetic acid was added twice instead of three times. For details, see IZIKOWITZ (1943) or FLODÉN (1955).

Accuracy of the method

The results obtained by earlier authors using double determinations for calculation – TROLLE used triple and quadruple determinations – are summarised below.

	Mean error of single determination	Mean error in per cent of values found
IZIKOWITZ, 1941	0.17 mg/100 ml	0.35
EEG-OLOFSSON, 1948	1.4 „	2.9
TROLLE, 1948	1.5 „	7.5
FLODÉN, 1955	0.18 „	0.36

In the present investigation all double determinations made between September 1956 and May 1957 were used in the calculation of the mean error of the single analysis. The results are presented below.

CSF sample	Total protein content (mg/100 ml)	No. of cases	Mean error of single determination (mg/100 ml)	Mean error in per cent of values found
1 ml	<50	30	1.00	3.0
1 ml	$\geqslant 50$	40	1.53	1.5
2 ml	<20	77	0.57	3.5
2 ml	$20<30$	64	0.55	2.3
2 ml	30—60	65	0.68	1.6
2 ml	>60	24	0.78	1.1

It is apparent that the order of the error of the method was dependent on the size of the CSF volume studied and on the amount of protein in the sample. The accuracy with the use of a sample of 2 ml was much better than with the use of 1 ml. With a volume of 2 ml the error of the method on determination of CSF samples with a protein content of less than 30 mg/100 ml was approximately constant (about 0.56 mg/100 ml), but when the amount of protein exceeded 60 mg/100 ml, it was about 40 per cent greater.

ELECTROPHORESIS OF CSF

A. CONCENTRATION OF CSF

Because of its low protein content — normally about one 300th of that in serum — the CSF must be concentrated before electrophoretic separation of the CSF protein fractions is possible. In the beginning of the present investigation use was therefore made of the method described by H. Esser et al. (1952) for pressure filtration through membranes impermeable to proteins. The concentration was carried out in a refrigerator at $+4°$ C in Thiessen apparatuses (Sartorius-Werke, Göttingen) and at about 3 atmospheres oxygen overpressure. Concentration of 6 ml of CSF — the volume usually employed for simple electrophoretic analysis of normal CSF

with negative protein reactions – required 16 hours. The ultrafiltrate was then always free of protein – tested with nitric acid, Esbach's reagent and sulphosalicylic acid.

During the later – and greater – part of the investigation the spinal fluid samples were concentrated by dialysis in collodium bags under negative pressure, as recommended by MIES (1953). Concentration by this method does not require quite such a large sample – for normal CSF about 4–5 ml – and usually takes only 4 to 6 hours.

In control experiments with serum diluted 1:300 and then concentrated, electropherograms of sera concentrated by both methods showed good agreement with that of the undiluted serum (Table 1). The slight decrease in the alpha$_1$-fraction, also observed by earlier investigators, no matter whether the CSF was concentrated by ultrafiltration (EWERBECK 1950) or by precipitation of the protein with

Table 1. *Comparison between undiluted serum and diluted serum after concentration.*

	Undiluted	*Diluted 1:300*	
		Concentration in dialysator	Concentration in collodium bag
Albumin	67.8	68.3	69.1
Alpha$_1$	3.9	3.4	3.4
Alpha$_2$	6.9	7.1	6.8
Beta$_1$	5.1	5.0	5.1
Beta$_2$	3.7	3.3	3.3
Gamma	12.7	12.9	12.2

Table 2. *Comparison of results obtained in a cerebrospinal fluid sample concentrated by two different methods.*

	Concentration in dialysator	Concentration in collodium bag
X-fraction	1.4	1.3
Albumin	74.7	74.3
Alpha$_1$	3.6	3.7
Alpha$_2$	4.4	4.2
Beta$_1$	5.0	5.3
Beta$_2$	3.2	3.1
Gamma	7.7	8.0

cold acetone (Bücher et al. 1952), has been ascribed to a presumed lability of this fraction (Ewerbeck 1950).

Table 2 compares the values found for fractions of a CSF sample concentrated by the two methods. It is clear that the electropherograms show good agreement.

B. PAPER ELECTROPHORESIS

Concentrated CSF samples were subjected to horizontal paper electrophoresis according to Cremer & Tiselius (1950) in a moist-chamber apparatus designed by C.-B. Laurell. The paper used was Whatman No. 1 for paper chromatography and the buffer consisted of a barbital buffer with calcium lactate of pH 8.6 and ionic strength 0.05 (Laurell, Laurell & Skoog 1956). A current of 120 v. D.C. was applied for 18–20 hours, and the environmental temperature was about +4° C.

The papers were afterwards placed in an oven at 120° C and allowed to dry in a horizontal position for 20 minutes. They were then stained for 10 minutes in bromphenol blue in methanol with 20 % $HgCl_2$ and rinsed for 10 minutes in each of 3 successive washing baths with 0.5 % acetic acid. The dried paper strips were cut into segments representing the different protein fractions and the dyes were eluted in 0.1 N Na_2CO_3 for 1 hour. The optical density was determined in a spectrophotometer (Beckman DU) at 595 mμ. After subtraction of the blank values, the extinctions of the fractions were summed up and the percentages of the various components were calculated.

Control of the method

Age of specimen

All the CSF samples were examined either immediately they were obtained or within 6 days. Samples not studied immediately were kept at +4° C, and the electrophoretic pattern did not differ from that of those samples studied immediately they were obtained. The

samples could be stored under sterile conditions for 2 months without any demonstrable changes in the electrophoretic pattern (Table 3).

Table 3. *Comparison of values found for a cerebrospinal fluid sample one day and 2 months after collection.*

	CSF one day after collection	Same sample studied two months later
X-fraction	0.7 *	0.6 *
Albumin	64.7	65.2
$Alpha_1$	4.0	4.1
$Alpha_2$	4.8	4.4
$Beta_1$	7.9	8.4
$Beta_2$	5.8	5.4
Gamma	12.1	12.0

* All values are means of double determinations.

Amount of specimen

In paper electrophoresis a certain amount of the albumin fraction lags behind along the path of migration. This "trailing" is constant and independent of concentration (HARDWICKE 1954, MACKAY et al. 1954, SCHULZ & HOLDCRAFT 1956 and KLATSKIN et al. 1956). The reduction in the albumin value will thus depend on the amount of the protein used. The volume of the samples should therefore not be too small. On the other hand, if the concentration on the paper is too high the protein will be understained. This decrease in dye-binding capacity is thought to be due to coagulum impermeable to the dye (HARDWICKE 1954, SCHULZ & HOLDCRAFT 1955 and KLATSKIN et al. 1956).

To determine the range of suitable amounts of the specimen, the method was tested with different amounts of serum, in which the volume used (0.02 ml) was kept roughly constant by dilution with physiologic saline.

The results are given in Table 4.

Thus, no significant difference was found in the distribution per cent of the protein fractions.

The volumes of spinal fluid examined were adjusted so that each

sample contained about 1 mg of protein as calculated with the aid of qualitative protein tests (Nonne, Pandy and/or Bisgaard). Nearly all of the electrophoretic patterns obtained also showed an albumin extinction value within the limits given above. A few samples showing extinction values not lying within this range were rejected.

Table 4. *Values obtained with varying sized aliquots of a given serum sample.*

Amount of serum	0.005 *ml*		0.01 *ml*	0.02 *ml*	0.03 *ml*	
Amount of protein	0.38 mg		0.76 mg	1.52 mg	2.28 mg	
		E.				*E.*
Albumin	71.9	1.08	71.5	70.9	70.5	11.0
$Alpha_1$	3.2	0.05	3.6	3.5	3.4	0.6
$Alpha_2$	9.1	0.14	9.7	10.0	9.9	1.6
$Beta_1$	4.2	0.06	4.5	4.0	4.5	0.7
$Beta_2$	3.0	0.04	3.2	3.4	3.5	0.5
Gamma	8.5	0.13	7.7	8.2	8.3	1.3

Electrophoresis of serum (total protein 7.6 g/100 ml). All figures represent means of three samples. The first and last columns include both the relative percentages and the corresponding extinction values (E). The serum samples in the two first columns — 0.005 ml, 0.01 ml, respectively — were all diluted with physiologic saline to 0.02 ml volume.

Age of dye solution

The dye solution, which is used several times, gradually becomes weaker. According to Schulz & Holdcraft (1955), freshly prepared solutions of the dye give a proportionately greater increase in the staining of albumin than of the globulin fractions and thus higher percentages of albumin than do solutions that have been used several times.

	Freshly prepared dye solution		6 month old, repeatedly used dye solution	
Albumin	73.9	*E.* 4.87	73.4	*E.* 4.07
$Alpha_1$	3.5		3.4	
$Alpha_2$	5.2		4.7	
$Beta_1$	5.5	*E.* 1.72	5.3	*E.* 1.45
$Beta_2$	2.5		2.4	
Gamma	9.4		10.8	

The left column shows a serum (P.I.) that was stained with fresh bromphenol blue solution and the right column refers to the same serum, which was stained with a 6 month old solution. Each column represents means of 3 determinations. The fresh dye solution thus gave a stronger coloration and higher extinction values than the old solution, the increase in colour of the various fractions being roughly proportional and thereby producing no obvious change in their distribution per cent, except for a slight relative understaining of the gamma-globulin.

In the present investigation the dye solutions were exchanged after being used for at most 3 months.

Accuracy of the method

A volume of 160 ml CSF, both normal and pathologic, was pooled. Volumes of 5 ml were concentrated in collodium bags and analysed in 4 different electrophoresis apparatuses. The papers were stained with the same dye solution, but on 3 different occasions. Results of the analysis of the 32 electropherograms are given below.

	Mean	Disperson	Disperson in per cent of fraction
X-fraction	3.0	0.37	12.3
Albumin	61.3	1.88	3.1
$Alpha_1$	4.5	0.65	14.5
$Alpha_2$	6.3	0.55	8.7
$Beta_1$	9.3	0.80	8.6
$Beta_2$	6.3	0.48	7.5
Gamma	9.2	0.98	10.6

Chapter II

THE CSF PROTEIN AND ITS FRACTIONS

TOTAL PROTEIN

The normal protein content of the CSF in adults is about one 300th of that of serum, the absolute values on record varying with the method of determination used (for references, see Izikowitz 1941). As to the normal CSF protein content in childhood, see page 39.

ELECTROPHORETICALLY SEPARATED PROTEIN FRACTIONS

The first to analyse the CSF electrophoretically was Hesselvik (1939), who used a Tiselius apparatus. The material consisted of unconcentrated CSF from two patients with general paralysis, and both patterns showed only an albumin and a gamma-globulin peak.

Using the same apparatus for electrophoretic analysis Kabat et al. (1942) investigated the CSF obtained in association with encephalography and ventriculography, concentrated by pressure dialysis to 0.4–1.5 g protein/100 ml. The electrophoretic pattern resembled that of serum and contained the same protein fractions. When the total protein content of the CSF was normal the authors were often unable to demonstrate alpha-globulin and fibrinogen, but in CSF samples containing an increased amount of total protein also these fractions were usually seen. In addition, some CSF samples showed a small, extra fraction with a somewhat higher rate of mobility than albumin. This fraction could not be demonstrated by electrophoresis of serum and was called the "X"-component by the authors.

Also using free boundary electrophoresis Scheid & Scheid (1944) observed the same fractions in CSF with increased total protein as in serum and with about the same distribution per cent. In normal CSF

concentrated to 0.1—0.2 g protein/100 ml Booy (1949, 1950) usually found only albumin, while pathologically changed CSF with increased total protein was found to contain also beta-globulin and gamma-globulin.

On electrophoretic analysis of normal CSF in a microapparatus according to Labhart & Staub, Labhart et al. (1951) found a separation of only albumin and total globulin, while Ewerbeck (1950), who used a similar apparatus (constructed by Antweiler) obtained a differentiation between the globulin fractions of normal CSF concentrated to 0.3—0.6 g protein/100 ml. Normal CSF was thus found to contain all of the electrophoretic fractions demonstrable in the serum.

The introduction of paper electrophoresis (Cremer & Tiselius 1950) made it possible to analyse the CSF with the use of much smaller quantities of CSF than before, and since then the CSF has been analysed practically always by this method. Quantitative data vary widely from one examiner to another owing to differences in the technique employed, *i.e.* electrophoretic and staining procedures, and above all the choice of method for quantitative evaluation. Numerical data from investigations in which direct densitometry of the filter paper strips has been employed are therefore not strictly comparable with data obtained by the dye elution technic.

With but few exceptions, however, the results of investigation on record show fairly good agreement. Independently of the choice of technique, the normal CSF electropherogram has certain typical features, which both qualitatively and quantitatively differ from the normal serum electropherogram.

X-FRACTION

The X-fraction first observed by Kabat et al. (1942) has proved to be a regular component of normal CSF. It has been called "Präalbumin" by V. Esser (1952) and "Vor-fraktion" by Bücher et al. (1952). Its concentration has been given by Steger (1953) as less

than 7 per cent of the total protein in lumbar CSF, as 7 to 13 per cent of cisternal CSF and as 13 to 20 per cent of ventricular CSF. Bauer (1956) found somewhat lower values for cisternal and lumbar CSF, but also in his material the relative concentration was highest in ventricular CSF and lowest in lumbar CSF.

If the total amount of protein is pathologically increased, the relative concentration of X-fraction is decreased (Gries, Aly & v. Oldershausen 1953), and in conditions accompanied by pronounced increase in the total protein in the CSF, such as in septic meningitis, the X-fraction is sometimes not demonstrable. On the other hand, no cases are on record in which the X-fraction of the CSF was pathologically increased (Bauer 1953). The increase observed by Hoch et al. (1952) and Tanaka (1956) in epilepsy is probably ascribable to the decreased total protein in that disease (Hohmann 1954), and possibly due to a functional disorder of the choroid plexuses with hypersecretion of ventricular CSF with its typical composition, *i.e.* low total protein level and relatively high X-fraction.

Fisk et al. (1951) made a detailed analysis of the fraction. The rapid component separated electrophoretically in a Klett–Tiselius apparatus was found to have a migration rate 1.3 times as fast as albumin and, on ultracentrifugation, to consist to 95 per cent of a homogenous component with the same sedimentation rate as albumin. In the 65 CSF samples studied the relative concentration varied between 3 and 17 per cent. In 9 cases a further and still more rapid component – called "X_1" by the authors – was isolated. Its relative concentration was, on the average, about 1 per cent of the total protein.

A similar analysis was carried out by Aly (1954) of the purified rapid component obtained on electrophoresis in a starch column. Ultracentrifugation and diffusion studies showed that the fraction was not homogenous but consisted of different-sized molecules, with molecular weights varying between 30,000 and 80,000.

The X-fraction has been conceived by some authors as a specific protein fraction of the CSF not occurring in serum (Fisk et al. 1951,

Plückthun et al. 1953 and Pieper 1954), while others are of the opinion that serum also contains this fraction in low concentration, but that it is masked by the other protein fractions, above all by the albumin (Esser 1952, Bücher et al. 1952 and Bauer 1956). A rapid "vor-fraction" has been demonstrated in serum from patients with tuberculosis (Seibert & Nelson 1942), in poliomyelitis (Booy 1953), and in essential hypoproteinemia (Schönenberg 1954). In normal serum and in normal plasma, respectively, it has been observed by Bücher et al. (1952), Hoch et al. (1953), Aly (1954a), Mumenthaler et al. (1957). By immunological analysis of serum in agar-agar, Grabar (1954) was able to demonstrate a protein fraction that migrated faster than albumin and that was serologically identical with pre-albumin in the CSF. These results were later confirmed by Schultze et al. (1956).

ALBUMIN

The relative concentration of albumin in the CSF is normally lower than in serum. It is lowest in ventricular CSF and highest in lumbar CSF (Steger 1953). Judging by the works of Fishman (1953) and Chou et al. (1955) – pages 26 and 29 – it may be regarded as established that the CSF albumin is identical with serum albumin.

ALPHA-GLOBULIN

In most investigations, no relative quantitative differences have been found between the alpha-fractions of normal CSF and of normal serum. However, the fractions have been described as less distinct and less well defined in CSF than in serum (Bauer 1953, Knapp 1955, Delank 1956 and Mumenthaler et al. 1957). Check investigations with diluted sera performed by the present author have shown that this is probably not due to the occurrence of any change during the concentration process but must instead be ascribed to a qualitative difference between the composition of the alpha-globulins in CSF and serum.

BETA-GLOBULIN

The relative concentration of the beta-globulin in normal CSF is higher than in serum. Like the X-fraction, the relative concentration is highest in ventricular CSF and lowest in lumbar CSF (Steger 1953). Two distinct beta-fractions can be distinguished in normal CSF, also with the use of barbituric acid buffer without any addition of calcium, while analysis with this buffer will usually show only one beta-fraction in the serum. The extra beta-fraction in the CSF has therefore been conceived by some investigators as a fraction specific of the CSF and possibly formed within the central nervous system (Esser 1952 and Bücher et al. 1952). In support of this assumption reference has been made to the not uncommon beta-globulin increase in the CSF in cerebral atrophy, disturbances of the cerebral circulation and similar conditions, in which increased catabolism of the brain substance is supposed (Esser 1952, Bauer 1953, and R. M. Schmidt 1956). Plückthun et al. (1953), could, however, show a serum $beta_2$-fraction occupying an electrophoretic position corresponding to the $beta_2$-globulin in the CSF and on electrophoresis with buffer containing calcium, a distinct $beta_2$-fraction is always obtained (Laurell et al. 1956).

GAMMA-GLOBULIN

The relative concentration of the gamma-globulin in the CSF is normally much lower than that in the serum. The CSF/serum quotient is given by some authors as ½ or less (Esser 1952, Aly 1952 and C. Schmidt et al. 1956), while others find higher quotients depending on differences in the technique employed. Steger (1953) and C. Schmidt et al. (1956) reported a lower gamma globulin content in ventricular CSF and cisternal CSF than in lumbar CSF. A division into two fractions is relatively often seen (Bücher et al. 1952, Delank 1956 and Mumenthaler et al. 1957).

SUMMARY

Electrophoretic analysis has shown the relative concentration of albumin and gamma-globulin of the CSF to be lower than that of serum and the relative concentration of the beta-globulin to be higher in the CSF. A fraction migrating somewhat faster than albumin is demonstrable on electrophoresis of the CSF, but usually not on such fractionation of serum. Finally, both alpha-fractions are less well defined than the corresponding fractions in serum. The differences between CSF and serum are most pronounced concerning ventricular CSF. They are less marked in CSF from other parts of the CSF space, especially from the most caudal parts.

Chapter III

PHYSIOLOGY OF THE CSF

Before discussing the CSF protein and its composition, it might be convenient first to dwell on the formation and reabsorption of the CSF.

FORMATION OF THE CSF

As early as 1664 WILLIS believed that the choroid plexus had something to do with the production of CSF. On the basis of histological investigations of the epithelium of the choroid plexus, FAIVRE (1854) and LUSCHKA (1855) arrived at the conclusion that the main site of production of the CSF was the choroid plexus. CUSHING's (1914) observation on the formation of drops on the surface of the exposed choroid plexus provided further support for this assumption. Other investigators, such as DANDY & BLACKFAN (1914) have confirmed this phenomenon. The validity of these conclusions is, however, debatable, because, as pointed out by DAVSON (1956) ... "the conditions are grossly abnormal, the intra-ventricular pressure being zero, so that any highly vascular region may be expected to exhibit exudation of extracellular fluid under these conditions".

DANDY's (1919) investigations on dogs with experimental hydrocephalus produced by obstruction of the aqueduct of Sylvius provide more acceptable evidence that the choroid plexus is the site of production of the CSF. He found that if the choroid plexus of one of the ventricules was removed, and both foramina of Monro were occluded, the operation resulted in a dilatation of the one ventricle with intact choroid plexus with collapse of the other.

In cases of idiopathic hydrocephalus internus, in which drainage was not obstructed, LUND (1956) found histologic changes in the

tissue of the plexus in the form of increased vascularisation, cavernous excavation of the plexus vessels and transformation of the normally cuboidal plexus epithelium to cylindrical epithelium, *i.e.* a histologic picture suggesting hyperfunction of the choroid plexus. These and similar observations made in cases of plexus papilloma, for example, and the favourable therapeutic effect of plexectomy in some cases of hydrocephalus internus also support the assumption that the choroid plexus is a site of production of CSF (LUND 1956).

Thus, although there has been wide agreement that CSF is produced by the choroid plexus, opinions have differed as to how the CSF is produced. Some authors are of the opinion that the formation of CSF is a physical phenomenon, and that CSF is an ultrafiltrate of blood plasma. MESTREZAT (1927), who put forward this theory claimed that the differences in the electrolytic composition of the CSF and plasma can be explained by differences in protein content. He based his opinion on experiments in which he submerged a collodium bag containing CSF from an experimental animal in blood plasma from the same animal. After the bag had been submerged in the blood plasma for 93 hours, he found no change in the CSF other than what could be ascribed to technical errors.

MESTREZAT's ultrafiltration theory has been criticized by STARY et al. (1929) and others. In experiments resembling MESTREZAT's, but performed on a larger scale, STARY et al. showed that the composition of the CSF differed essentially from an artificial ultrafiltrate from plasma, above all by its low potassium and phosphorus content and by its high magnesium content. On the basis of similar investigations DAVSON (1956) contended that the differences between the composition of the CSF and the plasma are such that they cannot be explained entirely by the Donnan equilibrium, and that the CSF must be produced in some way other than by dialysis from the blood plasma.

Adherents of the second theory of production of the CSF – the secretion theory – based their belief mainly on the glandular appearance of the plexus epithelium, described earlier by FAIVRE (1854)

and Luschka (1855). The concept of the choroid plexus as a gland is supported by the observation that the production of CSF is stimulated by some drugs such as pilocarpine, but inhibited by others such as atropine and scopolamine (Cappelletti 1901).

With reference to the histologic similarity between the choroid plexus and the glomeruli of the kidney, Purjesz et al. (1930) advanced the theory that the secretion of CSF resembles that of urine – a plasma dialysate formed in the choroidal villi is transformed by the plexus epithelium to a secretion with a composition characteristic of CSF. In support of their theory they refer to the observation that certain poisons produce similar histologic changes in the kidneys and in the choroid plexus; poisons damaging the glomeruli also damaging the choroidal villi, while toxic changes in the tubuli were accompanied by similar changes in the plexus epithelium. A similar view was put forward by Fleischmann (1920).

On the basis of dye-test experiments Broman (1949) concluded the existence in the plexus epithelium of a barrier between the blood and the CSF and that the selective passage of substances from the blood to the CSF is due to part of the plasma dialysate being actively transported back by the cells of plexus epithelium.

Clinical observations as well as experimental investigations thus strongly suggest that CSF is produced in the choroid plexus, probably by an active participation of the plexus epithelium.

While some workers in this field claim that all of the CSF is derived from the choroid plexus, *e.g.*, Kafka (1950), others assume that also other tissues may participate in the production. As early as 1855, Luschka concluded, on the basis of histologic observations, that the ventricular ependyma participates in the production of the CSF. Jacobi & Magnus (1925) claimed to have observed the formation of CSF from the ependyma of the lateral ventricles. Hassin (1925) on the basis of histo-pathologic observations concluded that the CSF is derived from the blood in the pial vessels and that the purpose of the choroid plexus is to transport waste products from the CSF.

With the aid of isotopic tracers SWEET & LOCKSLEY (1953) showed a rapid exchange of water and electrolytes between the blood and CSF independently of the choroid plexus. Each substance was exchanged at its special rate, varying from one segment of the CSF space to another. Water, for example, reached a state of equilibrium quicker in the cisternal CSF than in the ventricular CSF.

In similar investigations, in which heavy water was injected intravenously into normal human volunteers, BERING (1952) found that D_2O began to appear simultaneously in different parts of the CSF space. The concentration increased most rapidly in the cisterna magna, *i.e.* that part of the CSF space where the ratio between the surface area of nervous tissue and CSF volume was greatest. The "half-time", defined by the author as "the time required for the concentration to reach 50 per cent of the tracer-containing solution" increased with the age of the subject: in the cisterna magna it was 1.5–6 minutes; in the cerebral ventricles, 2–37 minutes; and in the lumbar subarachnoidal space, 7–38 minutes. Choroid plexectomy, which was performed on two patients with internal hydrocephalus, did not influence the D_2O exchange. Investigations with radioactive sodium and potassium (BERING 1955) gave similar results, although the exchange times were much longer than for D_2O. Neither then did plexectomy have any demonstrable influence on the exchange times.

In an investigation of the water exchange between the blood and the brain, BERING (1952) injected D_2O intravenously into dogs and then measured the radioactivity in different parts of the brain and the spinal cord. The experiments showed the presence of a free and very rapid turnover of water between the blood, brain and the spinal cord. The half-time in the cerebellum and in the grey substance of the cerebrum was 12 seconds, in the white substance it was 20 seconds, and in the spinal cord it was 25 seconds. Corresponding times for the CSF were: 8 minutes in the ventricles, 3 minutes in the cisterna magna, and 7 minutes in the lumbar subarachnoidal space.

Isotopic studies of recent years have thus shown that a rapid turnover of water and electrolytes occurs between the blood, brain and CSF. The exchange occurs by diffusion and mainly without participation of the choroid plexus. These observations, however, do not provide any explanation for the findings of DANDY and others in experimental hydrocephalus. It is therefore probable that CSF is really produced in the choroid plexus independently of the molecular exchange between the blood, brain and CSF.

There has also been divergence of opinion on the origin of the CSF protein. Many believe that it consists of serum protein that has, in one way or another, entered the CSF space. Others consider that the CSF protein is entirely or partly of meningeal or cerebral origin. Thus, LANGE (1923) expressed the view that the protein in the CSF is a disintegration product of the round cells normally occurring in the CSF. According to LANGE, the cells consist of desquamated epithelium from the walls of the CSF space, an opinion also shared by KAFKA (1952). MESTREZAT (1912), on the other hand, believes that both the protein and cells in the CSF are derived from the blood and that they pass through intercellular defects in the blood/CSF barrier, which is otherwise impermeable to colloids and corpuscular elements.

The introduction of electrophoresis and the use of isotope marked proteins implied the availability of better possibilities of investigating the origin of the CSF protein. Results obtained in the first electrophoretic experiments, which were made in a Tiselius electrophoresis apparatus, suggested that the CSF protein emanates from serum, because the electrophoretic patterns of CSF were found to be roughly the same as those of sera from the same persons. No definite qualitative difference could thus be observed between the protein of the two fluids by this method (KABAT et al. 1942 and SCHEID & SCHEID 1944). Further support for this theory of the serum as the origin of the CSF protein was provided by the observation that changes in the electrophoretic pattern of the serum protein are sometimes accompa-

nied by similar changes in the CSF protein. Thus, for example, an increase in the gamma-globulin in CSF was sometimes demonstrable in liver cirrhosis and veneral lymphogranuloma and a beta-globulin increase in beta myeloma, despite the absence of any neurological signs (KABAT et al. 1942 and SCHEID & SCHEID 1944).

WALLENIUS (1952), who used paper electrophoresis in his analysis of the CSF protein, also found the electrophoretic pattern to be approximately the same as that of serum. He concluded that the CSF and the serum proteins are normally identical. Later investigations with paper electrophoresis of the CSF protein have, however, shown qualitative and quantitative differences between serum protein and CSF protein.

The extra fractions "X" and "tau", which had not been observed earlier on serum electrophoresis, were conceived as specific of the CSF before their normal occurrence in serum had been established. It has also been questioned, whether these fractions might not originate in the actual central nervous system (FISK et al. 1951, and PLÜCKTHUN et al. 1953).

The observation made by ESSER (1952), BAUER (1953) and R. M. SCHMIDT (1956) of an increase in the beta-globulin in the CSF in certain cases of cerebral atrophy and disturbances of the cerebral circulation has been ascribed by ESSER (1952) and R. M. SCHMIDT (1956) to an increased catabolism of brain substance. That the proteins of the brain tissue consist mainly of beta-globulins has been shown by HOFMANN et al. (1956), for example. In distinction to the beta-globulins in the CSF, which have hardly any lipids, however, the beta-globulins of the brain tissue are rich in lipids.

Occasionally considerable increases have been observed in the normally relatively low gamma-globulin content of CSF in certain organic diseases of the central nervous system: neurosyphilis, multiple sclerosis and chronic meningitis and encephalitis. This increase is usually not accompanied by any corresponding change in the serum protein, and the gamma-globulin increase in CSF has been ascribed to a local production of antibodies within the tissues of the central

nervous system (KABAT et al. 1942, WALLENIUS 1952, BAUER 1953, STEGER 1953, VOLK et al. 1955 and DELANK et al. 1955).

No convincing evidence has been produced to show that protein is normally formed in the central nervous system. A cerebral or meningeal origin of the CSF protein has therefore been considered less probable. Serum protein has instead been widely regarded as precursor of CSF protein. Investigations with I^{131}-tagged albumin have also provided evidence in support of this assumption. Thus, FISHMAN (1953) found that I^{131}-tagged human serum albumin injected intravenously into dogs began to appear in the cisternal fluid within 40 minutes. After about 20 hours, the specific activity in the CSF was maximal and equal to that in the serum. "Specific activity" is to be understood as counts/second/mg total protein. In similar investigations on guinea pigs and mice, HOFMANN et al. (1955) showed that already a short time after the intravenous application of I^{131}-tagged albumin, the activity in the CSF corresponded to its protein content.

Thus, while such investigations made it probable that the albumin in the CSF originates entirely in the blood serum, no such evidence has been produced regarding the globulin fractions. However, FREUND's (1930) observation that the antibody titer of the CSF in passively immunized rabbits was about 1/300 of the serum titer, *i.e.* a strength corresponding to the gamma-globulin content of the CSF, might suggest that this fraction is also derived entirely from the blood serum.

Clinical observations and experimental investigations have thus made it probable that the CSF protein is normally entirely or mainly derived from the serum. As to where and how the protein enters the CSF space, several possibilities must be considered.

That the composition of the CSF protein varies with the level of the CSF space has been shown by electrophoretic investigations referred to earlier (page 16). Ventricular CSF differs most from serum in the composition of the protein, while lumbar CSF which also has the largest protein content differs least. Serum protein might therefore

normally be added to the CSF during its passage from the ventricles to the spinal subarachnoidal space, probably from the meningeal vessels (SCHÖNENBERG 1955, BAUER 1956, and SCHMIDT & MATIAR 1956). As shown by SCHALTENBRAND (1938), the admixture of colloids varies with the rate of flow – the slower the flow, the greater the colloid content.

By comparing the composition of the CSF in the cisterna magna with that in the lumbar subarachnoidal space, SCHMIDT & MATIAR (1956) could show that it is mainly the finely dispersed protein fractions in the serum that pass into the CSF. Thus, the increase in albumin was found to be five times as great as the gamma-globulin increase. It was assumed that the extent of this passage depended on the size of the surface between the serum and the CSF, thus greatest in the spinal subarachnoidal space. On intravenous injection of I^{131}-tagged albumin in man FISHMAN & RANSOHOFF (1955) also found the highest activity in the lumbar CSF and the lowest in the ventricular CSF.

The relatively protein-poor CSF in the ventricular system differs also in its protein pattern from cisternal and lumbar CSF. It is therefore probable that the proteins also enter in a different way.

WALLENIUS (1952), who was unable to demonstrate any definite electrophoretic differences in the protein pattern between the CSF and serum, explained the difference in protein content between ventricular fluid and lumbar fluid by the assumption that the permeability to proteins was less in the choroid plexus than in the meningeal vessels because of the lower number of intercellular leaks. DAVSON (1956), who in his investigations of the intraocular fluid found that very slowly penetrating substances presumably enter through intercellular pores, expressed the view that the proteins enter the ventricular fluid in a similar way via intercellular leaks in the choroid plexus.

Secretory activity of the choroid plexus, on the other hand, is supposed by BAUER (1956). On autoradiography of the choroid plexus of guinea pigs and mice, which had received I^{131}-tagged human al-

bumin intraperitoneally, HOFMANN et al. (1955) found numerous black points in the plexus cells. They interpreted this not as a sign of a secretory process in the strict meaning of the word, but instead as a result of an active transport function of the plexus epithelium suggested earlier by BROMAN (1949).

REABSORPTION OF THE CSF

KEY & RETZIUS (1870) observed that stained liquids injected subarachnoidally could afterwards be demonstrated in the Pacchionian bodies. This gave rise to the conception that all CSF absorption via these formations occurred to the large cranial venous sinuses. WEED (1914) who used the Prussian blue technique for his absorption studies, found coloured granules in the arachnoidal villi, of which the Pacchionian bodies are enlarged forms, as well as along the opticus and olfactory nerves. WEED concluded that the bulk of the CSF is reabsorbed directly by the blood through arachnoidal villi, but that a minor portion returns to the blood via the lymphatic perineural spaces.

HOWARTH & COOPER (1955), who reproduced Weed's experiments with the Prussian blue technique, however, found coloured granules also in the lumina of the small subarachnoidal vessels. Experiments with the use of radioactive dibromoprocaine (HOWARTH & COOPER 1949) also provided evidence of an absorption of CSF via capillaries and veins in the subarachnoidal space. Also other investigations, where radioactive substances were used, have shown that the reabsorption occurs mainly via capillaries and small veins, above all in the caudal part of the subarachnoidal space (ADAMS 1951 and EICHHORN 1957).

By diffusion into the parenchyma of the central nervous system, certain substances, especially water but also some electrolytes and lipoid soluble substances, come into direct contact with the capillaries of this tissue and can, in this way, be reabsorbed from the CSF space (SWEET & LOCKSLEY 1953, BERING 1954 and DAVSON 1956).

Reabsorption of colloids from the CSF space has been studied by HOWARTH (1952) and HOWARTH & COOPER (1955) with the aid of colloidal palladium deposited subarachnoidally in cats. They showed a direct absorption to the blood via the local intrathecal blood vessels, an absorption that was quicker in the cranial part of the subarachnoidal space than in the lumbar portion.

COURTICE & SIMMONDS (1951) and SIMMONDS (1952) injected plasma marked with Evans blue, which has a great affinity for proteins, into the cisterna magnum of cats and rabbits and found that already after 3–5 hours, by when the blood concentration of the coloured proteins reached its maximum, at least 20 per cent of the injected protein was absorbed by the blood stream. Only a small amount left the CSF space via the lymphatics.

On investigation of the absorption of I^{131}-tagged albumin, SWEET & LOCKSLEY (1953) found a very slow absorption on intraventricular application, but a much quicker absorption on subarachnoidal deposition of the test substance. The rôle played by the arachnoidal villi in the reabsorption of protein from the CSF was pointed out by the authors, who believed them to be analogous to the lymphatic vessels of the venous circulation. CHOU et al. (1955), who measured the absorption by the blood stream of I^{131}-tagged albumin injected into the lumbar subarachnoidal space, found that equilibrium between CSF and blood was reached after about 40 hours. The difference between this time and that found by FISHMAN (1953) — 20 hours — for the occurrence of equilibrium between the CSF and blood on intravenous injection of radioactive albumin was explained by the author by the assumption that albumin injected into the lumbar region does not reach equilibrium in the subarachnoidal space until after some 20 hours.

SUMMARY

A certain production of CSF occurs at the site of the choroid plexus. This CSF differs in composition from a pure plasma dialysate and is thus probably formed by a secretory or other active pro-

cess from the epithelium of the plexus. At the same time, however, certain substances, especially water, are in rapid exchange with the extracellular tissue of the brain and spinal cord.

Reabsorption of the CSF occurs partly via arachnoidal villi and Pacchionian bodies to the cranial venous sinuses, partly via intrathecal capillaries and veins and finally to some extent by way of lymphatics via the perineural spaces.

The protein in the CSF probably consists of serum protein, that has entered the CSF space. Protein is normally not formed within the central nervous system but may be under certain pathologic conditions. Normally, the serum protein enters the CSF space in two ways: partly via meningeal vessels above all of finely dispersed proteins especially albumin, and partly via the choroid plexus either by passage via intercellular leaks in the plexus epithelium or also through secretion or some other active process. The CSF formed by the choroid plexus is relatively poor in protein and has an electrophoretic pattern, that differs more from that of serum protein than does CSF fluid from other parts of the CSF space.

The reabsorption of the CSF protein occurs mainly directly to the blood stream, either via arachnoidal villi to the cranial venous sinuses or via intrathecal capillaries and veins. Reabsorption of protein via lymphatics is probably insignificant.

Part I

Studies of the CSF in normal subjects

Chapter IV

MATERIAL

The normal material consisted of 98 children, aged 0—13 years, admitted to the Children's Hospital, Lund, during the years 1955—1957. Of these children, 48 were below 1 year of age. None of them showed symptoms or signs of physical or mental maldevelopment and all were in a good general condition. No patients with evidence of infection such as fever, increased erythrocyte sedimentation rate or leucocytosis were accepted. Children who had signs of central nervous disease were not included either. All of the children below 1 year of age had weighed at least 2,500 grams at birth and are classified according to the reasons for admission in Table 5.

Table 5. *Reasons for admission.*

	No. of cases
Mother ill	17
Other social reasons	9
Feeding difficulties	7
Status post infectionem	7
Pylorospasm	4
Congenital heart disease, eczema, albuminuria, habitual vomiting	4
Total	48

The patients above 1 year of age had been admitted because of albuminuria, myalgia, diabetes mellitus, constipation, congenital heart disease, poor home conditions, etc.

In 15 cases the CSF was examined on more than one occasion. With the exception of 5 cases the interval between consecutive lumbar punctures was at least 14 days, and it was never less than 6 days. CSF was never collected on more than one occasion from children above 5 months. The numbers of examinations of the CSF in the

various age classes are given in Table 9. The total number of CSF samples examined was 123.

All of the samples were collected by lumbar puncture. As a rule, the puncture was made between the second and third lumbar vertebrae. Sometimes, however, the puncture was made between the first and second lumbar vertebrae, especially in the youngest infants. Puncture was never followed by undesired reactions. The volume of the sample varied with the age of the patient, 6 ml being collected from children below 6 months of age, 8 ml from children 6 months to 2 years of age, and 10—12 ml from children above this age.

The number of cells in the CSF sample was regularly determined in a counting chamber, and the samples were also studied by the methods of Nonne, Pandy and/or Bisgaard to assess the approximate amount of protein in the CSF. A more exact determination was then made of the total protein content by the method of Izikowitz, usually by double determinations (in 88 per cent of all determinations). Finally, the CSF protein was analysed with the aid af paper electrophoresis. 13 of the electrophoretic analyses and 2 determinations of the total protein content failed owing to technical mishaps.

In 25 of the normal children analysis also included electrophoretic examination of the serum. In 9 of these cases the studies were carried out on more than one occasion. Simultaneous electrophoresis of the CSF and of the serum was carried out on a total of 39 occasions, on 34 of which the samples emanated from children below 2 months of age. In addition, sera, but not CSF, from 52 children aged 6 weeks to 12 years were studied electrophoretically. This supplementary normal series of patients was selected according to the same criteria as the normal series proper and both series may therefore be regarded as comparable. The number of sera studied electrophoretically in each age group is apparent from Table 10.

In association with the collection of the material for examination of the CSF of patients with meningo-encephalitis, samples were also obtained from 12 adults in whom the clinical symptoms and diagnoses

were comparable to those in the normal series of children, *e.g.* myalgia, neurosis, vertigo not of central nervous origin, glaucoma, etc.

Analyses were also made of the CSF from 19 adult patients with acute infections of various types, such as influenza, fever of unknown cause, cystopyelitis, pharyngitis, otitis. The temperature and E.S.R. were usually increased, but the patients' general condition was good and apart from headache they had no symptoms suggesting any disease of the central nervous system.

In all 31 patients the number of cells in the CSF was normal – at most 3 per cmm. As is apparent from Table 12, both groups showed practically identical mean values for the total protein as well as for the different electrophoretically separated fractions. Both groups were therefore taken together as a single group, and although this "normal" material did not fulfil the same clinical requirements for normalcy as the normal series of children, it was uniform enough to be regarded as representative of adult age.

Chapter V

CELL CONTENT OF CSF IN NORMAL CHILDREN

Most investigators are in agreement regarding the cell content of CSF in normal adults, 3 cells per cmm usually being given as the upper normal limit (Neel 1928, Eskuchen 1930, Kafka & Samson 1929, Demme 1935).

Samson (1931) found the number of cells in the CSF to be the same in children above 6 months as in adults. During the first 3 months of life the cell-count has usually been described as higher than at any later period of life, though figures given vary widely from one investigator to another (Table 6). Samson (1931) and Otila (1948) found a higher cell-count in prematures than in full term newborns. Samson ascribes this entirely to an increased per-

Table 6. *Earlier investigations of the cell content (cells per cmm) of cerebrospinal fluid from normal infants.*

Age	Author	No. of cases	Minimum and Maximum values	Mean value
0—14 days	Waitz	50	5—20	—
	Stewart	—	28—34	—
	Samson	—	1— 6 2/3	3 1/3
	Wyers & Bakker	50	0—29	8 1/3
14 days—3 months	Stewart	23	16—24	23
	Samson	—	0— 5	3
	Otila	20	1— 4	—
3—6 months	Stewart	—	8—24	15
	Samson	—	0— 3 1/3	1 1/3

meability of the meninges in prematures, while OTILA expressed the view that intracranial hemorrhage during delivery might be a contributory cause.

In the present material the number of cells in the CSF during the first 3 months of life was found to be higher than during the following 9 months (Fig. 1). The individual variations were consider-

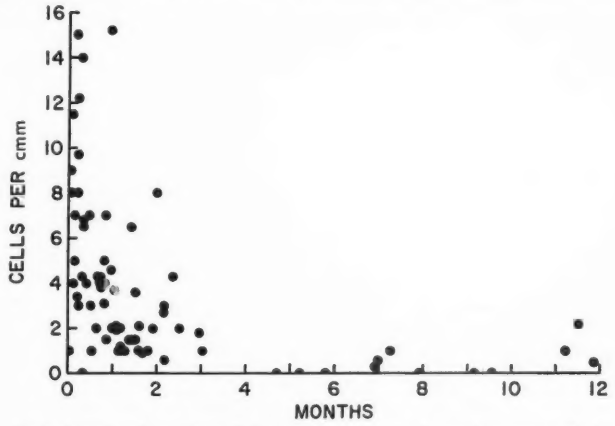


Fig. 1. Individual values found for cell content of cerebrospinal fluid from normal children during first year of life.

able, especially during the first 2 weeks of life. The number of cells in the CSF during this period varied between 0 and 15 per cmm (mean 7.5 per cmm), and the most common cells were polynuclear leucocytes (58 per cent of all cells). After the first year of life the lymphocytes were most common (62 per cent of all cells). The number of red blood cells in the CSF also varied widely, especially during the first 2 weeks of life (0–675 per cmm; mean value 120 per cmm). No certain relationship was found between the number of red blood cells and white blood cells ("cell-count") in the CSF.

The cell-counts found in the present material were higher throughout than those given by SAMSON (1931) and lower than those reported by STEWART (1928). Better agreement was found between the mean cell-count during the first week of life (8.1 per cmm) in

the present material and that noted by WYERS & BAKKER (1954) for the first day of life (8.3 per cmm).

The relatively large number of cells in the CSF during the first months of life is probably due mainly to a normal increase in the permeability of the meninges during this period permitting a greater passage of corpuscular elements from the blood stream into the CSF. Clinical and experimental data supporting this hypothesis are described on page 54.

It sounds unlikely that an intracranial hemorrhage or other meningeal irritation in association with delivery might be capable of producing an increase in the number of cells in the CSF for several months. As shown by SIMMONDS (1952), for example, subarachnoidally injected erythrocytes disappear from the CSF space within a few days. Similar observations have been made by NILSBY (1953) and CAESAR (1953) in post-puncture pleocytosis: aseptic reactions with increase in the number of cells in the CSF following lumbar puncture usually disappear within less than a week.

On the other hand, the neonatal increase in the number of cells in the CSF in certain cases may – as suggested by OTILA (1948) – be explained by a meningeal irritation in association with delivery, possibly as a consequence of hemorrhage in the meninges or adjacent structures. This theory will be discussed further in association with the account of the results of protein determinations during the neonatal period.

SUMMARY

In the present material the number of cells in the CSF was usually found to be higher during the first 3 months of life than during the remainder of the first year. Individual variations were considerable, especially during the first 2 weeks of life. No correlation was found between the number of red and white blood cells in the CSF. The main cause of the high number of cells in the CSF during the first months of life is probably a normally increased permeability of the meninges, possibly in combination with meningeal irritation sustained in association with delivery.

Chapter VI

PROTEIN CONTENT OF CSF IN NORMAL CHILDREN. EARLIER INVESTIGATIONS

On the basis of the results obtained on analyses of 200 CSF samples from healthy and sick children PFAUNDLER (1899) found the normal range of the total protein content of the CSF in infancy to be 20 to 40 mg/100 ml. The total protein content was determined by Brandberg's nitric acid test. He did not give the age distribution of his material.

On nepholometric determination of the total protein content of the CSF by Denis-Ayer's sulphosalicylic acid method, STEWART (1928) found the mean total protein content of 50 healthy children, aged 7 days to 12 years, to be 33 mg/100 ml. The single observations varied between 20 and 50 mg/100 ml. He found no relation between the total protein content of the CSF and the patient's age. Neither could he demonstrate any difference between the normal values in childhood and in adult age.

In 20 newborns with macroscopically clear CSF WAITZ (1928), who used SICARD & CANTALOUBE's volumetric method, found the total protein content of the CSF to vary between 30 and 100 mg/100 ml.

In his monograph on the CSF in childhood and based on results obtained in 1,500 examinations of CSF from healthy and sick children, SAMSON (1931) classified his normal material into 6 age groups. The normal total protein content of the CSF, as determined volumetrically by KAFKA–SAMSON's method, showed a distinct relation with the age of the children. During the first 14 days of life the total protein content varied between 40 and 80 mg/100 ml (mean

Table 7. *Earlier investigations of the total protein (mg per 100 ml) in cerebrospinal fluid from normal infants during first 14 days of life.*

Author	No. of cases	Minimum and Maximum values	Mean value	Method
Waitz (1928)	22	30—100	—	Sicard-Cantaloube: Volumetric determination after precipitation with trichloroacetic acid.
Samson (1931)	32	40— 80	60	Kafka-Samson: Volumetric determination after precipitation with picric acid.
Ujsághy (1936)	—	—	45	Ujsághy: Nephelometric determination after precipitation with semi-saturated ammonium sulphate in acidic environments.
Spiegel-Adolf et al (1954)	14	—	103	Denis-Ayer: Nephelometric determination after precipitation with sulphosalicylic acid.
Wyers & Bakker (1954)	50	33—162	96	"Nephelometric determination".

60 mg/100 ml). It then began gradually to decrease, and at 6 months of age the total protein content was roughly the same as that found by Kafka & Samson (1928) for adults. The mean total protein content was thus 20 mg/100 ml with the individual observations varying between 16 and 24 mg/100 ml (Tables 7 and 8).

In a similar investigation of 262 CSF samples from 58 healthy and 122 sick children Ujsághy (1936) determined the total protein content of the CSF nephelometrically after precipitation with semi-saturated ammonium sulphate in acidic environments. He also found a larger total protein content of the CSF during the neonatal period than during the rest of childhood, although the difference found was

Table 8. *Samson's and Ujsághy's values for total protein (mg per 100 ml) in cerebrospinal fluid from normal children.*

Age in months	Samson (1931)		Ujsághy (1936)
	Minimum and Maximum values	Mean value	Mean value
0—1/2	40—80	60	45
1/2—1	30—50	40	32
1—2	24—46	32	28
2—3	20—40	26	23
3—6	16—36	24	21
> 6	16—24	20	19

less than that given by Samson. The mean for the total protein during the neonatal period was also lower than Samson's (45 and 60 mg/100 ml, respectively), while both investigators gave the same total protein content for 6 months of age, namely 19 and 20 mg/100, respectively (Tables 7 and 8).

In association with his investigation of the CSF in prematures, Otila (1948) also studied the CSF in 20 full-term children, aged 2 weeks to 3 months. In these the total protein, which was determined by a colorometric method, based upon the xanthoproteic reaction, varied between 19 and 38 mg/100 ml (mean 30 mg/100 ml).

Spiegel-Adolf, Baird, Szekely & Wycis (1954) found a total protein content of the CSF of on the average 103 mg/100 ml in 14 normal newborns (nephelometric determination with sulphosalicylic acid), while 18 older children without signs of neurologic disease showed a mean of 28.5 mg/100 ml.

Wyers & Bakker (1954) studied the CSF from 50 normal children during the first day of life. The total protein content, which was determined nephelometrically, varied between 33 and 162 mg/100 ml (mean 96 mg/100 ml).

In order to estimate the amount of globulins in the CSF protein, attempts have been made, by adding magnesium sulphate or sodium

sulphate in varying concentration to the CSF, to precipitate the globulins or fractions of them. With this method SAMSON (1931) and UJSÁGHY (1936) found that during the first 3 months of life, especially during the neonatal period, the percentage of globulins in the CSF was greater than later in childhood.

The normal values given for the total protein content of the CSF in childhood vary considerably from one author to another. This lack of agreement might be explained by differences in the materials studied. Probably, however, the inaccuracy of the methods of determination used was of greater importance in this respect. As shown by IZIKOWITZ (1941), earlier quantitative methods based on volumetric, nephelometric or colorimetric determination of the protein in the CSF are so coarse or have such inherent systematic errors that their reliability for demonstrating the correct amount of total protein in the CSF must be seriously questioned. This has also been pointed out by later investigators (HINSBERG & GLEISS 1950, BAUER & ANGELSTEIN 1952 and EGGSTEIN & KREUTZ 1955). Particularly Kafka–Samson's volumetric method has been seriously criticised. Thus, DEMME (1935) who used the method in his analyses of the CSF stated that it cannot be regarded as a quantitative method in the strict sense of the word, but only permits a coarse estimation of the protein content of the CSF. Conversion of the "Teilstriche" to mg/100 ml is, according to him, not justified and gives a false impression of accuracy.

Determination of the globulin content of the CSF by salting out has also been seriously criticised. As shown by COHN et al. (1946), for example, the amount of globulins salted out by this method depends on the total protein concentration. In high protein dilutions e.g. normal CSF, a relatively smaller amount of globulins will be precipitated than in a more concentrated solution. The value of the globulin/albumin quotient of the CSF will thus depend to a great extent on the total protein content and might give a wrong impression of the true ratio between the globulins and albumin in the CSF

sample examined. It must therefore be questioned whether the high globulin/albumin quotient of the CSF during the neonatal period observed by Samson (1931) and Ujsághy (1936) might rather be due to the high protein content of the CSF in this age than to a relative increase in the globulin content.

Chapter VII

PROTEIN CONTENT OF CSF IN NORMAL CHILDREN. PERSONAL INVESTIGATIONS

The material examined was classified as follows according to the age of the child at the time of lumbar puncture.

Infants below 2 months were divided into 14-day groups – each of the first two weeks of life were accounted for separately. Children above 2 months of age were distributed among 3–4 month groups, according to the number of observations in the respective age classes. Children above one year of age were divided into 3 groups: one consisting of children up to 2 years of age, one of children aged 2–7 years, and the third 8–13 years.

The number of observations in each age class is given in Table 9.

RESULTS

The results of the examinations are given in Table 9 Figs 2–9.

The individual variation in the total protein content during the first 3 months of life was considerable. During the first 2 weeks of life the values observed varied between 33 and 119 mg/100 ml, and even in the subsequent age classes there was a considerable spread of the values (Fig. 2). After 3 months of age the range of variation was much narrower and remained so during the rest of childhood. Of 63 determinations of the total protein content in children aged 3 months to 13 years, 61 were 13–25 mg/100 ml. The mean total protein content in the various age groups (Fig. 3) showed a declining curve, which reached its minimum towards the end of the first year of life. After the second year of life the curve began to incline and continued to do so during the rest of childhood. Between the means

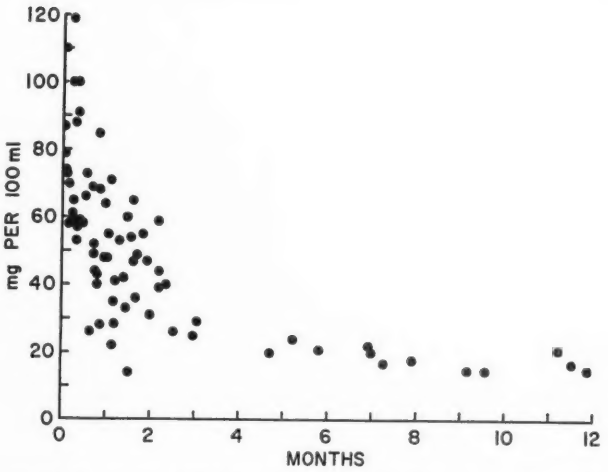


Fig. 2. Individual values found for total protein content of cerebrospinal fluid from normal children during first year of life.

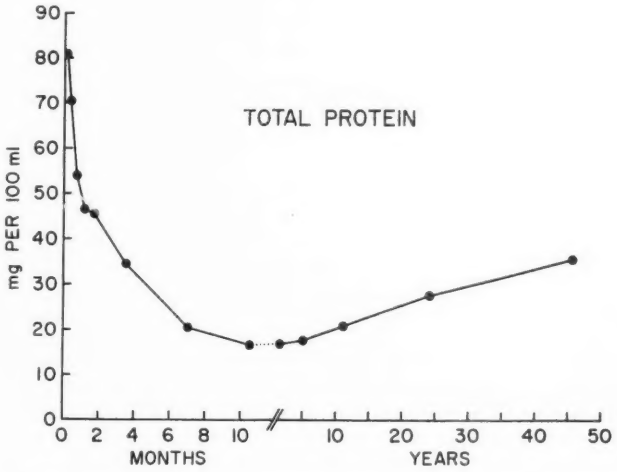


Fig. 3. Total protein content of cerebrospinal fluid from normal subjects of different ages. (Observe change of time scale at //.)

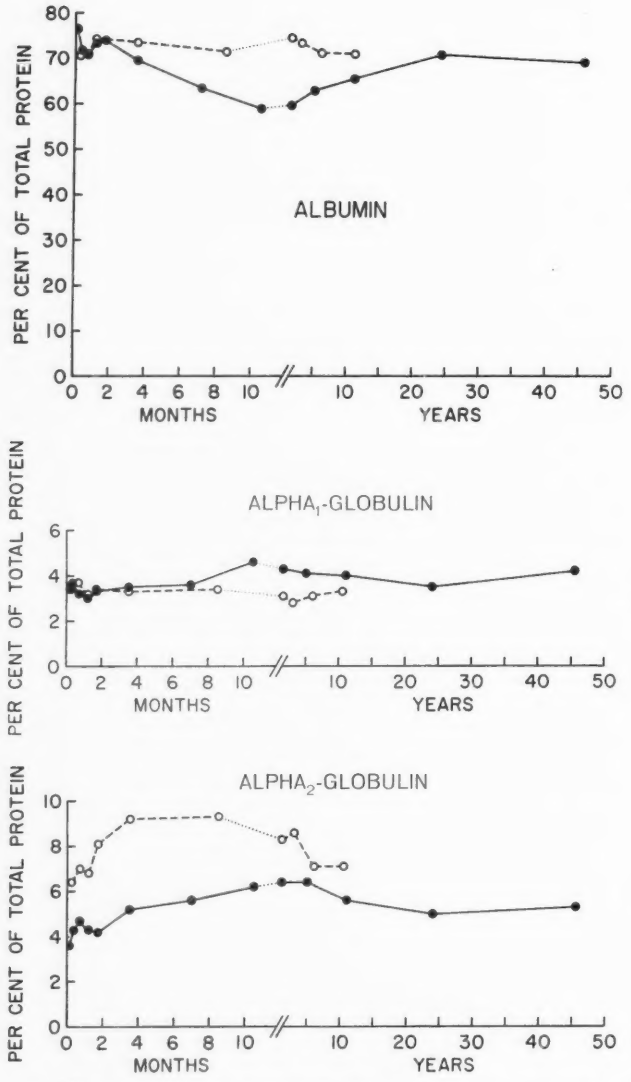
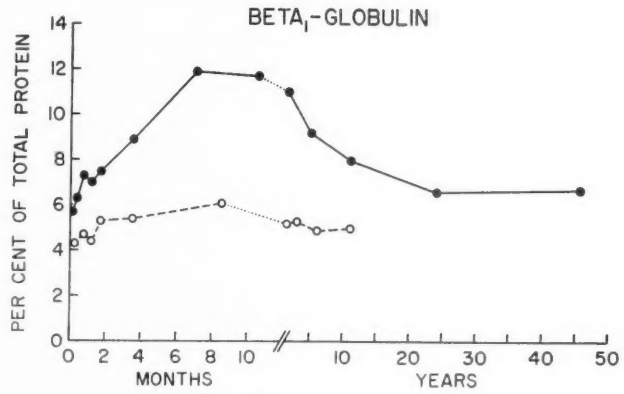
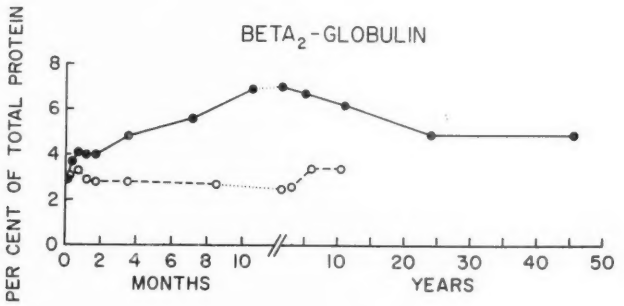


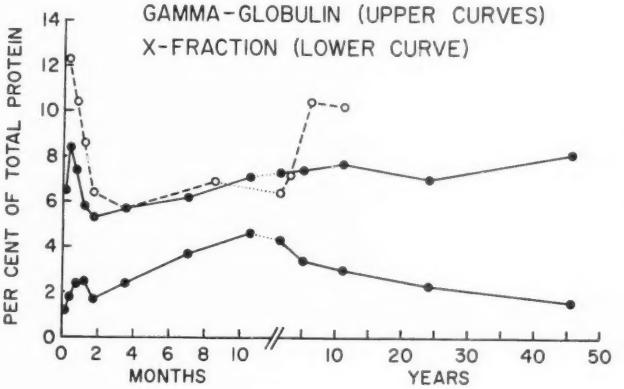
Fig. 4–9. Electrophoretically separated protein fractions of cerebrospinal fluid (●——●) and of serum (○----○) from normal subjects of different ages. (Observe change in time scale at //.)


BETA$_1$-GLOBULIN
PER CENT OF TOTAL PROTEIN
0
2
4
6
8
10
12
14
0 2 4 6 8 10
MONTHS
10 20 30 40 50
YEARS

7


BETA$_2$-GLOBULIN
PER CENT OF TOTAL PROTEIN
0
2
4
6
8
0 2 4 6 8 10
MONTHS
10 20 30 40 50
YEARS

8


GAMMA-GLOBULIN (UPPER CURVES)
X-FRACTION (LOWER CURVE)
PER CENT OF TOTAL PROTEIN
0
2
4
6
8
10
12
14
0 2 4 6 8 10
MONTHS
10 20 30 40 50
YEARS

9

Table 9. *Total protein (mg per 100 ml) and protein fractions of tot*

Age		Total protein	X	albumin	$alpha_1$	$alpha_2$	$beta_1$	$beta_2$	g
0— 6 days	n	11	10						
	m	80.9	1.2	76.7	3.4	3.6	5.7	2.9	
	s	20.8	0.95	3.5	0.99	0.81	0.76	0.58	
	e	6.3	0.30	1.1	0.31	0.26	0.24	0.18	
7—13 days	n	7	7						
	m	70.4	1.8	71.8	3.7	4.3	6.3	3.7	
	s	23.6	1.4	8.8	1.4	1.3	1.5	1.6	
	e	8.9	0.54	3.3	0.54	0.48	0.56	0.61	
14—27 days	n	13	13						
	m	53.9	2.4	70.7	3.2	4.7	7.3	4.1	
	s	17.8	1.5	3.4	0.88	0.78	1.6	0.94	
	e	4.9	0.43	0.94	0.24	0.22	0.45	0.26	
28—41 days	n	10	9						
	m	46.5	2.5	73.3	3.0	4.3	7.0	4.0	
	s	15.4	1.9	4.3	0.47	0.78	1.1	0.60	
	e	4.9	0.62	1.4	0.17	0.26	0.36	0.20	
42—59 days	n	11	11						
	m	45.6	1.7	73.9	3.3	4.2	7.5	4.0	
	s	14.2	1.4	7.4	1.5	1.1	1.8	1.0	
	e	4.3	0.42	2.2	0.45	0.32	0.55	0.31	
2— 4 months	n	9	7						
	m	34.8	2.4	69.6	3.5	5.2	8.9	4.8	
	s	12.0	1.0	2.8	0.99	0.87	1.1	0.75	
	e	4.0	0.39	1.1	0.37	0.33	0.42	0.28	

of the total protein in the 2–7 year age class (17.7 mg/100 ml) and the 8–13 year class (20.9 mg/100 ml) there was a significant difference ($2.7 \times e_D$).

The electrophoretic analysis of the CSF from the children showed that the composition of the CSF protein also varied with age. Thus, the relative concentration of albumin in the CSF was highest during the first week of life (Fig. 4) and lowest at the end of the first and

fractions of total protein) in cerebrospinal fluid from healthy children.

Age		Total protein	X	albumin	$alpha_1$	$alpha_2$	$beta_1$	$beta_2$	gamma
8 months	n	7	7						
	m	20.4	3.7	63.5	3.6	5.6	11.9	5.6	6.2
	s	2.4	0.60	3.7	0.79	0.94	1.3	0.78	1.3
	e	0.90	0.23	1.4	0.30	0.36	0.50	0.29	0.49
11 months	n	5	5						
	m	16.6	4.6	59.0	4.6	6.2	11.7	6.9	7.1
	s	2.6	2.0	3.6	0.40	0.89	1.7	0.90	0.82
	e	1.2	0.90	1.4	0.18	0.40	0.76	0.40	0.37
23 months	n	8	7						
	m	16.8	4.3	59.7	4.3	6.4	11.0	7.0	7.3
	s	2.7	1.6	9.4	0.99	1.4	2.4	1.7	2.0
	e	0.98	0.60	3.6	0.37	0.54	0.90	0.65	0.74
7 years	n	21	17						
	m	17.7	3.4	62.9	4.1	6.4	9.2	6.7	7.4
	s	3.1	0.94	7.6	1.1	1.6	1.9	1.9	2.7
	e	0.68	0.23	1.8	0.26	0.38	0.47	0.45	0.66
13 years	n	19	17						
	m	20.8	3.0	65.6	4.0	5.6	8.0	6.2	7.7
	s	4.1	0.83	5.6	0.80	1.1	1.5	1.4	2.1
	e	0.95	0.20	1.4	0.19	0.27	0.36	0.34	0.51

during the second year of life. It then began to increase, and in the 2—13 year age class the relative amount of albumin in the CSF was higher than in the 9—23 month class (Table 13). The X-fraction, alpha- and beta-globulins showed the opposite variation with low values during the neonatal period and maximal values at 1—2 years of age. As is apparent from Figs. 4—9, the differences between serum and CSF in most of the fractions were greatest for the 9 month to 2 year age class. Both below and above this age class a certain decrease in the differences was noted, especially in newborns.

The relative concentration of the gamma-globulin in the CSF, like that in the serum, was higher during the neonatal period than during

Table 10. *Protein fractions (per cent of*

Age		albumin	$alpha_1$	$alpha_2$	$beta_1$	$beta_2$	gamma
0—13 days	n	14					
	m	70.7	3.4	6.4	4.3	3.0	12.1
	s	4.6	0.72	1.3	0.63	0.89	2.8
	e	1.2	0.19	0.34	0.17	0.24	0.75
14—27 da	n	10					
	m	71.0	3.7	7.0	4.7	3.3	10.4
	s	5.5	0.84	1.6	0.66	0.80	2.4
	e	1.7	0.27	0.51	0.21	0.25	0.77
28—41 days	n	7					
	m	74.2	3.2	6.8	4.4	2.9	8.6
	s	3.1	0.74	0.88	0.70	0.26	1.7
	e	1.2	0.28	0.33	0.26	0.10	0.64
42—59 days	n	6					
	m	74.1	3.4	8.1	5.3	2.8	6.4
	s	4.6	0.77	0.96	0.45	0.49	3.1
	e	1.9	0.32	0.39	0.18	0.20	1.3
2— 4 months	n	12					
	m	73.5	3.3	9.2	5.4	2.8	5.7
	s	4.8	0.39	2.4	0.88	0.69	1.8
	e	1.4	0.12	0.72	0.28	0.21	0.56

the rest of infancy (Fig. 9). As in the serum, there was then a rapid and marked decrease. At 6–8 weeks of age the relative concentration of the gamma-globulin of the CSF again began to increase and continued to do so throughout the rest of infancy. The pronounced increase in the gamma-globulin of the serum at about 4 years of age was not accompanied by any such increase in the CSF. Therefore in children above 4 years of age the serum differed distinctly from the CSF in the relative concentration of the gamma-globulin.

total protein) in serum from healthy children.

Age		albumin	$alpha_1$	$alpha_2$	$beta_1$	$beta_2$	gamma
5—11 months	n	9					
	m	71.5	3.4	9.3	6.1	2.7	6.9
	s	2.8	0.50	1.5	0.74	0.52	1.7
	e	0.91	0.17	0.51	0.25	0.17	0.57
12—23 months	n	8					
	m	74.6	3.1	8.3	5.2	2.5	6.4
	s	3.0	0.37	1.0	0.74	0.61	1.6
	e	1.1	0.13	0.36	0.26	0.22	0.58
2— 3 years	n	10					
	m	73.4	2.8	8.6	5.3	2.6	7.2
	s	2.5	0.51	0.87	0.70	0.55	1.8
	e	0.79	0.16	0.27	0.22	0.17	0.56
4— 7 years	n	8					
	m	71.2	3.1	7.1	4.8	3.4	10.4
	s	4.6	0.43	1.6	0.82	0.91	2.6
	e	1.6	0.15	0.57	0.29	0.32	0.91
8—13 years	n	6					
	m	71.0	3.3	7.1	5.0	3.4	10.2
	s	4.1	0.77	1.3	0.71	0.37	2.2
	e	1.7	0.31	0.53	0.29	0.15	0.88

Electrophoretic analysis of the CSF protein during the first 6 weeks gave results differing from the general trend in the later course. The results are therefore accounted for separately below.

During the first week of life the relative concentration of the albumin in the CSF (Fig. 4) was greater than in the serum, which is normally not seen later in life. In order to estimate the situation in the individual cases, a comparison was made between the albumin concentration in serum and CSF in samples collected at the same

time. As is apparent from Table 11, during the first 10 days of life the relative concentration of the albumin in the CSF was usually higher than in the serum. Between 15 and 30 days the relationship was less irregular, and after the first month of life the relative concentration of the albumin in the serum was with but few exceptions higher than in the CSF, a difference normally persisting throughout the rest of life. The difference between the three groups was significant.

Table 11. *Comparison between the relative albumin content of cerebrospinal fluid and of serum from same individuals. Healthy children aged 0—9 months.*

Group	Age in days	No. of cases		Mean of Difference	Error of Mean
		Total	CSF albumin higher than serum albumin		
I	0—10	14	11	+3.6	1.0
II	16—30	13	6	−0.5	1.0
III	>30	12	2	−6.0	1.5

Difference I—II $=4.1=2.9\times e_D$
Difference II—III $=5.5=3.0\times e_D$

During the second to fourth weeks of life the relative concentration of the albumin in the CSF decreased markedly (Fig. 4). This decrease was followed by a new increase during the second month of life, after which the relative concentration of the albumin continuously decreased until the end of the first year. The other protein fractions of the CSF showed a more or less converse tendency (Figs. 5—9).

To form an opinion of the CSF protein changes in the individual cases during the first months of life, those cases were studied in which electrophoresis of the CSF had been performed both between the ages of 7—27 days and 28—59 days. The material included only 7 such cases. Of these, 5 showed a higher relative albumin concentration during the latter period.

DISCUSSION

Judging by the results presented above, the normal protein content of the CSF during the first 3 months of life is much higher than that given earlier by SAMSON (1931) and UJSÁGHY (1936). The range of variation during this period is probably also much wider than that given by these authors. This discrepancy is probably ascribable to the inaccuracy of the methods used earlier. It might be mentioned that in a series of normal adults and with the same method as that used in the present investigation IZIKOWITZ (1941) also found higher values for the total protein content and a wider individual range than had been found in earlier investigations.

The total protein values in the neonatal period found by SPIEGEL-ADOLF et al. (1954) and by WYERS & BAKKER (1954) are not strictly comparable with those noted in the present investigation because they only studied 1-day old children.

In the discussion of the physiology of the changes in the amount and composition of the CSF protein during childhood the origin of the CSF protein and its passage into the CSF space must be considered. As mentioned earlier, the CSF formed in the ventricular system is characterised by a low total protein content, a low relative albumin concentration and high relative concentrations of beta-globulins and X-fraction. By the admixture of serum protein entering the CSF via the meningeal or subarachnoidal vessels, the protein content of the fluid changes in amount and composition during its passage to the lumbar subarachnoidal space.

Comparison of the results for different periods of childhood shows that in children in the 9 month—2 year age class the lumbar CSF resembles ventricular CSF more closely with regard to the content and composition of its protein than during any other period of life.

The higher protein content and the more serum-like composition below and above this age class are probably due to a relatively greater admixture of serum protein, either via the choroidal plexus or via

the meningeal or subarachnoidal vessels during the passage of the CSF from the ventricles to the lumbar subarachnoidal space.

That the blood-CSF barrier in infants is more permeable than otherwise during childhood has long been known. Thus, an increased permeability to uranine during the first year of life was shown by LEONOW (1927), while KRUSE (1929) found an increased permeability to bromine and KAFKA (1953) to immune bodies during early infancy. In the same way the normal yellow colouring of the CSF during the neonatal period has been ascribed mainly to an increased permeability of the blood-CSF barrier in respect to bilirubin in this age. Hemorrhage in the CSF space in association with delivery with subsequent hemolysis has been considered of less importance in this respect (GLASER 1930 and OTILA 1948). In investigations of the bilirubin content of the CSF in Rh-immunised newborns STEMPFEL & ZETTERSTRÖM (1955) were able to show a significant correlation between the indirect bilirubin and the total protein of the CSF. Thus, while the increased admixture of serum protein to CSF below 9 months of age is probably due to an increased meningeal permeability during this age, changes in the total amount and composition of the CSF protein after 2 years of age cannot be explained in this way. No experimental evidence has been produced to support the assumption that the permeability of the blood-CSF barrier increases with age.

The apparently increased admixture of serum protein might, on the other hand, be explained by a decreased production of CSF by the choroidal plexus. The production of CSF in different ages was studied by BOURDILLON, FISCHER-WILLIAMS, SMITH & TAYLOR (1957) by comparing the rate of entry of bromide and radiosodium into the CSF. These two substances differ from one another in the way they pass from the blood stream into the CSF space. While the bulk of radiosodium accompanies the fluid formed by the choroidal plexus, the bromide is retained to a certain extent by plexus epithelium, so that the CSF formed in the ventricles will be very poor in bromide. Bromide in the CSF is therefore derived mainly from a

meningeal admixture. On the basis of their results BOURDILLON et al. concluded that the production of CSF by the choroidal plexus will decrease with increasing age.

More difficult to explain are the changes in the pattern of the CSF protein during the first 6 weeks of life. Irregularities in the otherwise fairly smooth curves for the different protein fractions may be due to irregularities of the material examined. The individual variations in this age were considerable, and no statistically significant difference was found between the relative albumin concentration in the CSF at 7–27 days and 28–59 days. The observation that the same alterations in the protein pattern also occurred in most of the individual cases examined do, however, suggest that the alterations observed were true changes.

During the first 6 weeks of life the serum gamma-globulin level decreased markedly (Fig. 9) and the albumin increased moderately (Fig. 4), while the other protein fractions showed more irregular curves (Figs. 5–8). A certain parallelism was found between the changes in the protein fractions in the serum and CSF during this period. The latter changes were therefore probably secondary to the former.

The high relative albumin content of the CSF at birth was not accompanied by any similar change in the serum. However, as will later be apparent, the relative albumin concentration in the CSF is higher than in the serum also during the intial stage of acute meningitis and meningo-encephalitis. In such cases the increase in the albumin of the CSF is probably due to an altered meningeal permeability owing to the infection with subsequent increase in the passage of serum protein into the CSF space. That the albumin thereby enters to a greater extent than the other protein fractions can probably be explained by the relative smallness of its molecules. One might possibly imagine a similar physiologic sequence of events in normal newborns. As mentioned earlier (page 38), the trauma of delivery may imply mechanical irritation of the meninges with an "aseptic meningitis" as a consequence.

In this respect it might be mentioned that on electrophoretic analysis of the CSF protein of 22 newborn infants ARNHOLD & ZETTERSTRÖM (1958) found that "... the relationship between the concentrations of the individual fractions in serum and cerebrospinal fluid was (also) fairly constant and similar to that reported for other ages". From this the authors concluded, that all fractions penetrated the blood-CSF barrier at the same rate. Check-analysis of their data, however, revealed that the relative albumin concentration was higher in the CSF than in the serum.

As will later be apparent, in acute abacterial meningo-encephalitis the relative concentration of the albumin in the CSF decreases after the first week of the disease, while the globulin fractions, especially the gamma-globulin, increase. This increase is probably due to a retarded re-absorption to the blood. It is possible that this might also help to explain the earlier discussed irregularity in the curves for the protein fractions of the CSF in children below 2 months of age. However, the present investigation produced no convincing evidence to support this assumption.

SUMMARY

The results of the determinations of the total protein content and of the electrophoretic analyses of the CSF in normal children may be summarised as follows.

In newborns the average protein content of the CSF was much larger and the individual variations much wider than later in childhood. After a successive decrease the mean total protein content dropped to its lowest level between the ages of 9 months to 2 years, at the same time as the individual spread decreased markedly. At 2 years of age the total protein content of the CSF again successively increased and continued to do so during the rest of childhood.

Of the electrophoretically separated protein fractions of the CSF albumin in its variation with age showed a distinct parallelism with the total protein. The mean value found for the albumin concentra-

tion of the CSF – expressed as a percentage of the total protein – was thus highest in the neonatal period and lowest between the ages of 9 months and 2 years, after which it again began to increase. The other protein fractions of the CSF, except the gamma-globulin, showed roughly the converse variation.

The physiologic basis for these changes in the amount and composition of the CSF protein in different ages is probably a variation in the relative amount of the admixture of serum protein during passage of the CSF from the ventricles to the lumbar subarachnoidal space. The increased addition observed below 9 months of age is probably due to an increased permeability of the blood-CSF barrier. The changes occurring after 2 years are probably related to a relatively decreasing function of the choroidal plexus with increasing age.

Immediately after birth the relative albumin concentration in the CSF was higher than in the serum. The physiologic basis of this is presumably mechanical irritation of the meninges in association with delivery and subsequent increase of passage of serum protein into the CSF space, especially of the relatively finely dispersed albumin molecules.

Chapter VIII

PROTEIN CONTENT OF CSF IN "NORMAL" ADULTS

Of the 31 adult "normals" investigated, 17 were at most 30 years of age; the remainder, above this limit. The mean ages in these 2 groups were 22 and 46 years.

RESULTS

The total protein content was as a rule somewhat higher in the older group (Table 12 and Fig. 3). In this group the mean total protein content was 36 mg/100 ml, the individual observations varying between 23 and 46 mg/100 ml. In the lower age group the total protein content was on the average 28 mg/100 ml and the individual observations ranged between 16 and 41 mg/100 ml. Although the material was small — 10 and 15 observations respectively — the difference between the means of the two groups was statistically significant ($2.8 \times E_D$).

The electrophoretic analyses showed only significant differences between the two age groups with respect to the relative concentrations of X-fraction and alpha-globulin. (Table 12 and Figs. 4–9.)

DISCUSSION

Judging by the results obtained for the "normal" material, the total protein content of CSF in adults is much higher than in children above 2 years of age (Table 13). The relative albumin concentration is also higher in adult age, while the X-fraction, the beta-globulins and to a certain extent the alpha-globulins are relatively lower in adults than in children.

Also between the age groups of 8–13 years and 17–30 years

Table 12. *Total protein (mg per 100 ml) and protein fractions (per cent of total protein) in cerebrospinal fluid from adults. Group I consists of healthy subjects. Group II consists of infected subjects. The differences between the mean values for subjects 17—30 years and subjects above 30 years are given in units of standard error of the difference ("t"-value).*

		Total protein	X	albumin	$alpha_1$	$alpha_2$	$beta_1$	$beta_2$	gamma
Group I	n	7	12						
	m	33	1.9	70.2	3.7	5.2	6.6	4.7	7.7
Group II	n	18	19						
	m	30	2.1	69.9	3.9	5.1	6.7	5.0	7.3
All subjects	n	25	31						
	m	31	2.0	70.0	3.8	5.1	6.7	4.9	7.5
A. Subjects	n	15	17						
17—30 years	m	27.6	2.3	70.7	3.5	5.0	6.6	4.9	7.0
	s	6.7	0.77	5.3	0.55	1.1	1.3	1.4	1.6
	e	1.7	0.18	1.3	0.13	0.27	0.32	0.35	0.39
B. Subjects	n	10	14						
31—59 years	m	35.6	1.6	69.2	4.2	5.3	6.7	4.9	8.1
	s	7.6	0.70	4.0	0.91	1.2	1.1	1.3	1.7
	e	2.4	0.19	1.1	0.24	0.33	0.29	0.36	0.45
Difference A—B	t	2.8	2.6	0.9	2.7	0.9	—	—	1.8

statistically significant differences were found in the total protein content and in the relative concentration of albumin and beta-globulin.

In an investigation of mixed "normal" adult material consisting of persons with respiratory infections and preoperative cases during spinal anesthesia MÜLLER, JAWORSKY, SILVERMAN & ELWOOD (1954) also found the total protein content of the CSF to increase with age. In the highest age group – patients above 60 years – they sometimes found values exceeding 60 mg/100 ml. The total protein content was determined nephelometrically by the method of Denis-Ayer.

Like the differences between the various age periods in childhood the differences between children and adults in the content and com-

Table 13. *Total protein (mg per 100 ml) and protein fractions (per cent of total protein) in cerebrospinal fluid from healthy children and from adults. The differences between the mean values are given in units of standard error of the difference ("t"-value).*

		Total protein	X	albumin	alpha$_1$	alpha$_2$	beta$_1$	beta$_2$	gamma
I. Children	n	13	12						
9—23 months	m	16.7	4.4	59.4	4.4	6.3	11.3	7.0	7.2
	s	2.6	1.7	7.2	0.70	1.2	2.1	1.4	1.5
	e	0.71	0.49	2.1	0.23	0.34	0.60	0.40	0.44
II. Children	n	40	34						
2—13 years	m	19.1	3.2	64.2	4.0	6.0	8.6	6.4	7.5
	s	3.9	0.90	6.7	0.92	1.4	1.8	1.6	2.4
	e	0.63	0.15	1.1	0.16	0.24	0.31	0.28	0.41
III. Adults	n	25	31						
17—59 years	m	30.8	2.0	70.0	3.8	5.1	6.7	4.9	7.5
	s	8.0	0.81	4.7	0.80	1.2	1.2	1.4	1.7
	e	1.6	0.14	0.85	0.16	0.21	0.22	0.25	0.31
Difference I—II	t	2.7	3.2	2.1	1.2	0.7	4.2	1.0	0.4
Difference II—III	t	7.9	5.5	4.0	1.0	2.7	5.4	4.1	—

position of the protein in the CSF might most likely be explained by variations in the admixture of serum protein to the CSF. These are, however, probably only apparent and due to a decrease in the CSF production by the choroidal plexus with increasing age.

SUMMARY

The average total protein content of the CSF in the adults studied was much higher than in children above 2 years of age. Also between older children, aged 8–13 years, and young adults, aged 17–30 years, the difference was appreciable. In addition the adults above 30 had a higher total protein content in the CSF than younger adults. Of the various protein fractions of the CSF, the relative albumin concentration was higher in adults than in children above 2

years, while the relative concentrations of X-fraction and beta-globulin were smaller.

The most probable explanation of this change in the amount and composition of the CSF protein in adult ages is, like the changes in children of different ages, a relative decrease in the CSF production by the choroidal plexus with age with subsequent increase in the relative amount af admixture of serum protein.

Part II

Studies of the CSF in acute abacterial meningo-encephalitis

Chapter IX

DEFINITION OF THE DISEASE AND DESCRIPTION OF THE MATERIAL STUDIED

Acute abacterial meningo-encephalitis is to be understood as an acute infection with meningeal or cerebral symptoms, increase in the number of cells in the CSF, sterility of the CSF as judged by microscopy and culture, and usually of brief benign course (WALLGREN 1925). It is, however, difficult to define the disease exactly, because the etiology is often obscure. Earlier the disease was conceived as one of a definite etiology (WALLGREN 1925), probably viral, but later virologic and serologic investigations have shown that the syndrome may be produced by a variety of different agents (WALLGREN 1951, ALM 1951, NILSBY 1953, PETTE 1954, JOHNSSON 1956).

The disease may be caused by certain viruses, protozoa, Rickettsiae, spirochetes and fungi. In addition cases of meningo-encephalitis are on record where toxic or allergic origin may be suspected. Although it has been possible by culture and serologic methods to detect the causal agent in an increasing percentage of the cases, there still remains a group of unknown etiology.

The present material consisted of 119 patients who had been admitted to the Children's Hospital, Lund, and to the Departments of Epidemic Diseases, Lund, Malmö and Hälsingborg, during the period November, 1955, to May, 1957. The clinical diagnoses in these cases were "meningo-encephalitis acuta", "serous meningitis", "lymphocytic meningitis", "viral meningitis" and "mumps meningitis". The cases are classified according to their etiology in Table 14.

The diagnosis mumps meningo-encephalitis was usually made on clinical or epidemiologic grounds, as was meningo-encephalitis after herpes zoster and varicellae. The other infections were diagnosed viro-

Table 14. *Composition of material.*

Diagnosis	No. of cases	Per cent
Unknown	61	51
Mumps	48	40
ECHO-virus	4	3
Adeno-virus	2	2
Herpes Zoster	2	2
Mononucleosis	1	1
Varicellae	1	1
Total	119	100

logically and/or serologically. The table does not, however, give an exact picture of the relative frequencies of the different etiologic agents. Thus, for example, the true frequency of adeno-virus and ECHO-virus infection in the present material was probably higher than that suggested by the table, because it was only during the latter part of the present investigation that laboratory facilities were available to diagnose these conditions. Nevertheless, the frequencies given agree with those presented by earlier authors (HAUSSMANN 1955, HENNESSEN 1956 and STRÖM 1956).

The material included no cases of meningo-encephalitis after measles or vaccination. Nor did it comprise any cases of post infection meningo-encephalitis, such as after pneumonia, dyspepsia or whooping cough. In the group of unknown etiology (61 cases) poliomyelitis could be excluded by virologic and/or serologic investigations in 51 cases (84 per cent) and Coxsackie in 21 cases (34 per cent).

The age and sex distribution of the material is given in Table 15. The distribution did not show such a preponderance of the males in the mumps meningo-encephalitis group as did those reported by KILHAM (1949) and STRÖM (1956). Among the cases of meningo-encephalitis not related to mumps males were somewhat preponderant, but in view of the size of the material, this might be ascribable to chance. Thus no difference in frequency of the disease was found with sex in the present material.

As to the frequency of disease in the various age groups, mumps

Table 15. *Age and sex distribution of material.*

	No. of cases	Age range in years	Mean age in years	Males		Females	
				No.	%	No.	%
Children:							
mumps meningo-enc.	33	1—14	8	15	45	18	55
other types of meningo-enc.	27	1—13	9	16	59	11	41
Adults:							
mumps meningo-enc.	15	15—56	24	7	47	8	53
other types of meningo-enc.	44	15—58	33	25	57	19	43
Total	119	—	—	63	53	56	47

meningo-encephalitits was most common during the early years of life Thus mumps meningo-encephalitis represented more than half of the total number of children, but only one fourth of the adult material. In addition, the average age of the adults with mumps meningo-encephalitis was about 10 years lower than that of the remainder. The material included no patients below one year of age.

A total of 256 CSF samples were examined. In 230 the examination included determination of the total protein content and electro-

Table 16. *Number of cerebrospinal fluid samples analysed in material.*

	I	II	III	Total
Children:				
mumps meningo-enc.	61	3	5	69
other types of meningo-enc.	63	—	6	69
Adults:				
mumps meningo-enc.	21	1	3	25
other types of meningo-enc.	85	3	5	93
Total	230	7	19	256

I. Determination of total protein content and electrophoresis.
II. Determination of total protein content only.
III. Electrophoresis only.

phoretic analysis – in the remaining 26 only one of these two methods was used (Table 16). In more than half of the total material (68 cases) CSF was studied electrophoretically during the initial stage of the disease (within one week of onset) and on one occasion or more during the later stage of the disease. In the remaining 51 cases electrophoresis of the CSF was performed either within the first week or on one occasion or more later (Table 17).

Table 17. *Material classified according to time of collection of cerebrospinal fluid for electrophoresis.*

	I	II	III	Total
Children:				
mumps meningo-enc.	20	7	6	33
other types of meningo-enc.	14	2	11	27
Adults:				
mumps meningo-enc.	8	5	2	15
other types of meningo-enc.	26	8	10	44
Total	68	22	29	119

I. Collected both during and after first week of disease.
II. Collected only during first week of disease.
III. Collected only after first week of disease.

Chapter X

CHANGES IN THE CSF AND THEIR RELATION TO THE ETIOLOGY OF THE DISEASE AND THE AGE AT ONSET

CELL CONTENT AND TOTAL PROTEIN CONTENT

As found by earlier investigators (KAHLMETER 1956, HENNESSEN 1956 and others) in similar series, the number of cells in the CSF at the time of onset of the disease in the present material was as a rule higher in mumps meningo-encephalitis than in other types of meningo-encephalitis. Among the children this difference was striking, a cell-count of less than 100 cells/cmm during the first 3 days of the disease having been noted among only 12 per cent of the patients with mumps meningo-encephalitis, as against 60 per cent of the remainder (Fig. 10). The mean number of cells in the CSF during the first week of the disease also differed between the two groups (Table 18). The difference between the means was $2.5 \times$ ED.

Also among the adults the number of cells in the CSF at the time of onset was usually higher in patients with mumps meningo-encephalitis (Fig. 10). These two groups did not, however, differ from one another regarding the means for the first week of the disease owing to a few single observations with very high cell-counts (about 1500/cmm) among the patients with meningo-encephalitis not related to mumps.

Examination of the CSF after the first two weeks of the disease showed roughly the same number of cells in children with mumps meningo-encephalitis as in the other children (Table 18 and Fig. 11). A certain tendency to a further decrease in the cell-count during the fourth to fifth week of the disease was, however, observed among the children with mumps meningo-encephalitis. In adults the cell-count during the corresponding period was usually higher than in

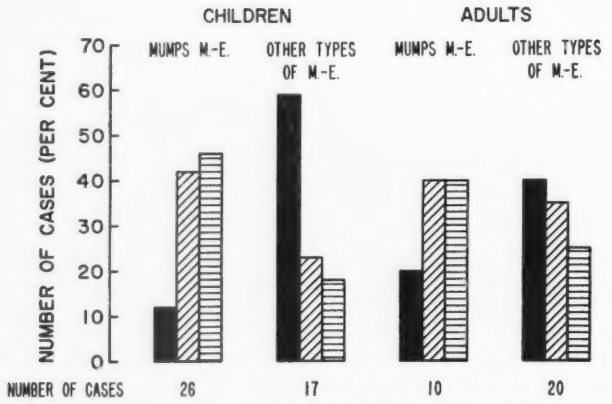


Fig. 10. Cell content in CSF during first 3 days of acute abacterial meningo-encephalitis. Distribution within different classes of cell-counts.

■ Cases with <100 cells/cmm
▨ Cases with 100—400 cells/cmm
▤ Cases with >400 cells/cmm

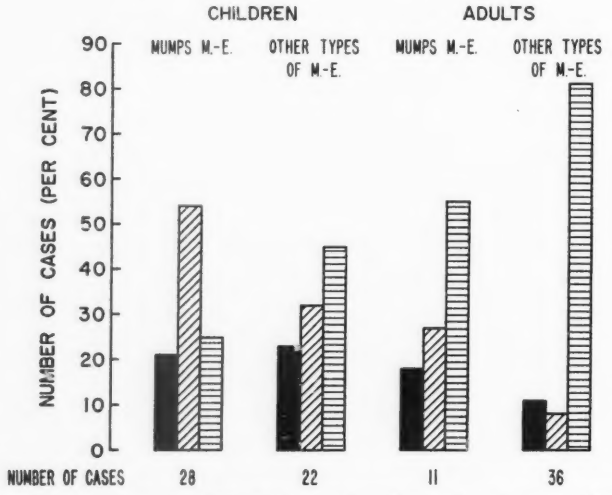


Fig. 11. Cell content in CSF during 3rd—5th weeks of acute abacterial meningo-encephalitis. Distribution within different classes of cell-counts.

■ Cases with <4 cells/cmm
▨ Cases with 4—9 cells/cmm
▤ Cases with >9 cells/cmm

Table 18. *Cell content (cells/cmm) in cerebrospinal fluid during different phases of acute abacterial meningo-encephalitis.*

		Days after onset		
		<7	14—20	21—34
Children:				
mumps meningo-enc.	n	31	15	15
	m	290	11	7
other types of meningo-enc.	n	21	12	14
	m	141	9	10
Adults:				
mumps meningo-enc.	n	13	5	6
	m	239	14	14
other types of meningo-enc.	n	38	21	17
	m	267	24	36

children (Table 18 and Fig. 11). In addition, during the third to fifth week of the disease the number of cells in the CSF was lower in adults with mumps meningo-encephalitis than in the other adults. The difference between the means of the two groups was $2.6 \times e_D$[1] and the difference in distribution according to cell-count was appreciable (Fig. 11).

During the first week of the disease the total protein content of the CSF was higher in children with mumps meningo-encephalitis than in the other children (Table 19). The difference between the mean values of the groups was, however, only $2.0 \times e_D$. Of the adults, those with meningo-encephalitis not related to mumps had a higher protein content of the CSF. The difference between the means of the groups (28 mg/100 ml$=3.2 \times e_D$)[1] was too large to be ascribed entirely to the difference in the mean ages. In addition the spread of the individual values was wider among the patients with meningo-encephalitis not related to mumps (standard deviation=44 and 15 mg/100 ml, respectively).

After the first 2 weeks of the disease the total protein content of the CSF in the children decreased to roughly the same level in both groups, while among the adults a difference persisted which was too

[1] e_D was calculated by the formula $\sqrt{e_1^2+e_2^2}$.

Table 19. *Total protein content (mg/100 ml) in cerebrospinal fluid during different phases of acute abacterial meningo-encephalitis.*

		Days after onset		
		<7	14—20	21—34
Children:				
mumps meningo-enc.	n	27	14	15
	m	44	25	25
other types of meningo-enc.	n	13	11	13
	m	31	23	25
Adults:				
mumps meningo-enc.	n	13	4	4
	m	53	40	35
other types of meningo-enc.	n	34	19	16
	m	81	51	55

large to be ascribed entirely to difference in mean ages. The mean total protein content of the CSF in the 2 adult groups 3—5 weeks after onset of the disease was 38 and 53 mg/100 ml, respectively (difference$=3.5\times e_D$).[2] The standard deviation 3—5 weeks after the onset was 7.7 mg/100 ml for adults with mumps meningo-encephalitis and 19.9 mg/100 ml for the remainder.

These results will be discussed in the following section in connection with the account of the CSF changes and the clinical symptoms.

ELECTROPHORETICALLY SEPARATED PROTEIN FRACTIONS

In earlier investigations of acute bacterial meningitis and tuberculous meningitis electrophoretic analysis during the acute stages of the diseases usually showed a serum-like composition of the CSF protein with increased relative albumin and gamma-globulin concentrations and a decreased relative beta-globulin concentration (v. Oldershausen, Aly & Gries 1953, Bellieni & Biagini 1954, Koch 1956 and Matiar & Schmidt 1957). During the later course of the

[2] e_D was calculated by the formula $\sqrt{e_1^2+e_2^2}$.

diseases a further increase in the relative concentration of the gamma-globulin in the CSF was noted, particularly in chronic meningitis such as tuberculous and fungus meningitis.

In corresponding investigations of acute abacterial meningo-encephalitis of viral origin, the results obtained have been less uniform. In most cases the relative concentration of albumin in the CSF has been found to be decreased, even in the acute stage of the disease (Caspani & Sticca 1953, Rossi & Schneider 1953, Andres 1955 and Schönenberg 1955) most frequently in association with an increase in gamma-globulin. Matiar & Schmidt (1957) found an increase in the relative concentration of the albumin in the CSF during the initial stage of lymphocytic meningitis, although less pronounced than what had been noted in acute bacterial meningitis and tuberculous meningitis. No quantitative data were given. Non-conclusive electropherograms showing no definite tendency have been described by Gomirato Sandrucci (1954), Schmidt & Knittel (1957) and others.

RESULTS

In the present material electrophoretic analysis of the CSF protein during different stages of acute abacterial meningo-encephalitis in the two age-groups studied revealed no definite difference between mumps meningo-encephalitis and meningo-encephalitis of other origin. Therefore in the classification according to age both groups are taken together irrespective of the cause of the meningo-encephalitis. The results of the examination are given in Table 20.

Both in the children and in the adults the relative concentration of albumin in the CSF increased markedly during the initial stage of the disease. During this period the other protein fractions were below normal. During the further course a successive normalisation was observed. The gamma-globulin, which was also decreased during the initial stage of the disease, increased to levels above normal during the following weeks.

In an attempt to study the relation between the CSF protein and

Table 20. *Protein fractions in cerebrospinal fluid (per cent of total protein) during different phases of acute abacterial meningo-encephalitis (c=children, a=adults).*

Days after onset	No. of cases		X		albumin		$alpha_1$		$alpha_2$		$beta_1$		$beta_2$		gamma	
	c	a	c	a	c	a	c	a	c	a	c	a	c	a	c	a
0— 1	23	17	1.4	0.9	75.4	74.9	3.5	3.4	4.2	4.3	5.9	6.0	3.6	3.6	5.9	6.8
2— 3	16	16	1.7	1.0	71.8	73.8	3.6	3.6	5.2	4.5	6.7	6.4	4.1	3.9	6.9	6.9
4— 9	9	17	2.1	0.7	66.2	71.8	4.2	3.5	5.6	5.0	7.3	5.9	5.5	4.0	9.1	9.4
12—20	28	24	2.5	1.2	66.4	71.0	3.7	3.4	5.9	4.6	8.4	6.4	6.1	4.7	6.9	8.7
21—30	25	21	2.4	1.4	66.7	70.7	3.7	3.2	5.5	4.7	8.1	6.6	5.8	5.0	7.8	8.5

the serum protein during different stages of the disease, electrophoretic analyses were made of CSF as well as of serum from 5 children (mean age 8 years) admitted to the Children's Hospital, Lund, because of acute abacterial meningo-encephalitis. The results of these examinations are given in Table 21.

Table 21. *Electrophoresis of cerebrospinal fluid and serum from 5 children with acute abacterial meningo-encephalitis within 3 days of onset and 2—3 weeks later (C=cerebrospinal fluid, S=serum).*

Time after onset	X	albumin		$alpha_1$		$alpha_2$		$beta_1$		$beta_2$		gamma	
	C	C	S	C	S	C	S	C	S	C	S	C	S
0—3 days	1.2	71.2	67.2	3.7	4.0	6.3	10.5	6.9	5.0	4.2	3.7	6.4	9.6
2—3 weeks	2.3	59.4	70.0	4.3	3.7	7.5	8.0	9.7	5.1	7.7	3.0	9.1	10.1

In these 5 cases the relative concentration of the protein fractions of the CSF varied in the same way as in the remainder of the material. Thus, the high relative concentration of albumin during the initial stage of the disease decreased markedly 2—3 weeks later with a simultaneous relative increase in the concentration of the globulin fractions.

During the initial stage the serum protein pattern showed changes typical of an acute infection: increased $alpha_2$-globulin and slightly decreased albumin. Two to three weeks later the pattern was again normal. During the initial stage of the disease the mean value of the relative concentration of the albumin in the CSF was higher than in the serum. Also in the individual cases the relative concentration of the albumin in the CSF during the first 4 days was always higher than in the serum. On the other hand, the relative concentration of the albumin in the CSF 2—3 weeks later was regularly lower than that of the serum albumin.

In a further 9 cases — patients cared for at the Children's Hospital, Lund, for acute abacterial meningo-encephalitis, simultaneous electrophoretic analyses of the CSF and of the serum were performed during the initial stage (2 cases) or after the first two weeks of the di-

sease (7 cases). Those 2 examined during the initial stage showed a higher relative concentration of albumin in the CSF than in the serum, while those examined later during the course of the disease had a higher relative albumin concentration in the serum than in the CSF, with the exception of 2 cases. The size of the difference in the total material is given in Table 22.

Table 22. *Comparison between the relative albumin content of cerebrospinal fluid and serum from 14 children with acute abacterial meningo-encephalitis. +indicates that the relative concentration of albumin was higher in the cerebrospinal fluid than in the serum, and — that it was lower.*

Time after onset	No. of cases		Range of difference	Mean of difference	Standard deviation
	Total	CSF-alb. higher			
0—3 days	7	7	+ 2.3 — + 7.6	+ 4.4	2.1
>13 days	12	2	— 15.8 — + 2.9	— 8.6	6.8

DISCUSSION

The changes in the composition of the protein of the CSF during different stages of acute abacterial meningo-encephalitis are probably due to altered permeability of the meninges. In the acute stage of the disease the changes that CSF primarily formed in the ventricles normally undergoes on its passage to the subarachnoidal space will be pathologically augmented by the markedly increased admixture of serum protein. It cannot, however, be excluded that the composition of the CSF formed in the ventricles may be abnormal because of increased permeability of the blood-CSF barrier in the choroidal plexus. It is probably easiest for the finely dispersed protein fractions, especially albumin, to enter the CSF space, so that the main change in the protein pattern during the acute stage of the disease will consist of a pronounced increase in the albumin. Similar views on the cause of the increase in the albumin of the CSF in inflammatory diseases of the meninges have been put forward by Matiar & Schmidt (1957).

During the subsequent course of the disease, after the meningeal permeability has begun to recover, the proteins are re-absorbed. The relatively large molecular and probably less readily absorbed gamma-globulin will thereby show somewhat hypernormal values for some time. As previously pointed out, there is a certain similarity to the normal changes in the electrophoretic pattern of the CSF protein during the neonatal period.

SUMMARY

At the time of onset of the disease the cell content of the CSF was usually higher in mumps meningo-encephalitis than in meningo-encephalitis of other origin. The difference was particularly striking among children. After the first 2 weeks of the disease no difference was found between the two groups in the children series, while adults with mumps meningo-encephalitis at this stage had a lower cell-count than the remainder.

At onset as well as during the later course of the disease the total protein content of the CSF was higher in adults with meningo-encephalitis not related to mumps than in those with mumps meningo-encephalitis.

During the initial stage of the disease a pronounced increase was noted in the relative concentration of the CSF albumin which thereby exceeded that of serum. At the same time the other protein fractions of the CSF were decreased. During the further course the pattern gradually became normal although the relative gamma-globulin concentration was above normal for some time afterwards.

The abnormalities in the electrophoretic pattern of the CSF protein are probably due to disturbed meningeal permeability. During the initial stage of the disease serum albumin may pass into the CSF space more readily than the remaining serum protein fractions. The increase in the gamma-globulin concentration during the later course of the disease might be explained by a relative retardation of the reabsorption to the blood stream.

Chapter XI

CHANGES IN THE CSF AND THEIR RELATION TO THE CLINICAL PICTURE

Earlier investigations have shown no parallelism between the changes in the CSF and the intensity and duration of the symptoms in acute abacterial meningo-encephalitis. This discrepancy between the CSF findings and the clinical symptoms has been found both in mumps meningo-encephalitis (Montgomery 1934, Jersild 1942, Levison & Thordarson 1942, Holden, Eagles & Stevens 1946, Fox & Grotts 1949, Bieling & Koch 1952, Bowers & Weatherhead 1953, and Copaitich & Mingrino 1955) and in meningo-encephalitis of other etiology (Jacobsson & Holmgren 1949, Solomons, Markman & West 1953, Steen 1954, Dein 1954 and v. Oldershausen 1957). Neither has any correlation been found between the CSF changes during the acute stage of the disease and sequelae in the form of persistent symptoms (Oldfelt 1949). Attempts have been made mainly to correlate the clinical symptoms with the cell content of the CSF.

PERSONAL STUDIES OF DISEASE AT TIME OF ONSET

In order to determine whether any correlation could be demonstrated in the present material between the CSF findings and the clinical symptoms at time of onset, the cases in the 4 groups described earlier (page 67) were classified according to the severity of the clinical symptoms at the time of onset.

To *group I* were assigned all patients with mild symptoms and signs. These patients had only slight meningeal symptoms in the form of mild headache, giddiness, nausea or neckstiffness for at most

3 days. The temperature, unless ascribable to some other factor, was above 39° for at most one day, and above 38° for at most 5 days.

Group II consisted of those patients who had either pronounced or persistent – more than 3 days – meningeal symptoms of the type described above. This group also included patients with cerebral symptoms in the form of decreased level of consciousness, convulsions or pareses.

RESULTS

It is clear from Table 23 that the clinical symptoms at time of onset were by no means so severe among patients with mumps meningo-encephalitis as among the remainder.

Among the patients with mumps meningo-encephalitis no difference was found between the CSF cell-count of those with mild symptoms and those with severe clinical symptoms (Table 23, Fig. 12). Among the remainder, however, the two groups differed with

Table 23. *Cell content (cells/cmm), total protein (mg/100 ml) and gamma-globulin (per cent of total protein) in cerebrospinal fluid during the first 5 days of acute abacterial meningo-encephalitis of varying severity at onset.*

		No. of cells		Total protein		Gamma-globulin	
		I	II	I	II	I	II
Children:							
mumps meningo-enc.	n	19	12	17	10	15	12
	m	286	298	38	55	6.0	7.1
other types of meningo-enc.	n	9	12	6	7	6	10
	m	101	172	28	34	5.7	7.0
Adults:							
mumps meningo-enc.	n	7	6	7	6	7	5
	m	252	172	58	45	7.4	6.6
other types of meningo-enc.	n	8	30	8	26	7	25
	m	133	321	55	84	7.9	7.1

I. Patients with mild symptoms at onset of disease.
II. Patients with moderate to severe symptoms at onset of disease.

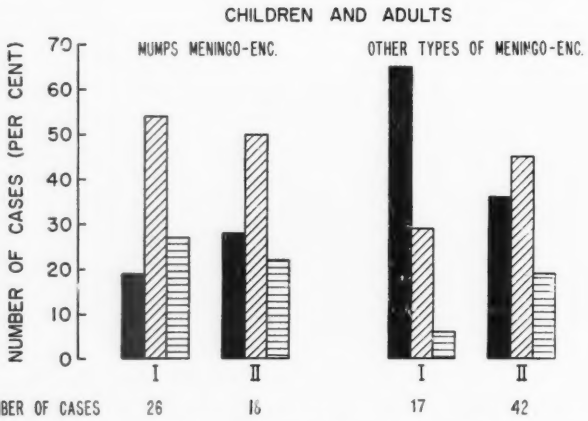


Fig. 12. Cell content in CSF during first 5 days of acute abacterial meningo-encephalitis of varying severity at onset. Distribution within different classes of cell-counts.

I. Cases with mild symptoms at onset of disease.
II. Cases with moderate to severe symptoms at onset of disease.

■ Cases with <100 cells/cmm
▨ Cases with 100—400 cells/cmm
▤ Cases with >400 cells/cmm

respect to the distribution among different classes of cell-counts (Fig. 12).

The mean value found for the total protein content of the CSF in children with mumps meningo-encephalitis was somewhat higher in group II than in group I. The difference between the means was, however, not significant. Of adults with meningo-encephalitis not related to mumps, the total protein content of the CSF was on the average, higher in those belonging to group II. The difference between the means of the groups was statistically significant ($3.2 \times e_D$).[1] In addition the individual spread in group II was much wider than

[1] In the determination of the significance of differences given in this chapter e_D was calculated according to the formula: $\sqrt{e_1^2+e_2^2}$.

in group I (standard deviation=37 and 17 mg/100 ml, respectively). In both groups the mean age was roughly the same (31 and 33 years). In the two remaining small groups – children with meningo-encephalitis not related to mumps and adults with mumps meningo-encephalitis – group I was not found to differ from group II regarding the total protein content of the CSF.

As to the electrophoretically separated protein fractions of the CSF, the only difference between group I and group II was found in the relative concentration of gamma-globulin. The other protein fractions showed roughly the same relative concentration in both groups and are therefore not described here. As is apparent from Table 23, the gamma-globulin value in children was somewhat higher in group II than in group I, while the reverse was found in adults. The differences between the means of the groups were, however, not significant.

PERSONAL STUDIES TWO WEEKS AFTER ONSET OF DISEASE

During the post-acute stage of acute abacterial meningo-encephalitis the symptoms may vary widely from patient to patient. Some are symptom-free within a few days, while others are troubled for a long time by fatigue, headache, concentration difficulties, irritability, etc. In an attempt to demonstrate any difference between the CSF findings in patients who had prolonged symptoms and those who were soon symptom-free, the material was classified according to the severity of the symptoms 14 days after onset.

The first group (A) included those patients who were completely free of symptoms or who had only slight trouble in the form of spells of very mild headache or tiredness. They were all classified as healthy 7 weeks after onset.

The second group (B) consisted of those patients in whom the symptoms were more severe, though not incapacitating. In this group the patients were classified as healthy within 3 months of onset.

To the third group (C), those patients were assigned who had pronounced and persistent symptoms in the form of tiredness, headache, dizziness, loss of memory, concentration difficulties, irritability, abnormal intolerance of light and sound. Also patients with such symptoms of moderate severity but persisting for more than 3 months were included in this group.

This classification cannot, of course, be more than approximate. Data on which the classification was based were obtained from the records from different hospitals written by different examiners and were therefore not strictly comparable. In addition the border-line between groups A and B must be relatively diffuse. On the other hand, group C probably includes fewer border-line cases because these patients were more or less incapacitated by their symptoms.

RESULTS

Within none of the groups previously defined with respect to age and etiology (page 67) was any definite correlation found between the severity of the clinical symptoms at the time of onset of the disease and the severity of the symptoms two weeks later. The mean age of the adults with meningo-encephalitis not related to mumps was somewhat higher in groups B and C (32 and 34 years) than in group A (26 years). In the rest of the material the mean age was roughly the same in all 3 groups.

Table 24 shows that, practically speaking, all of the children with mumps meningo-encephalitis were completely free of symptoms after the first 2 weeks, while many of the children with meningo-encephalitis of other origin still had symptoms. Also among the adults the frequency of clinical symptoms was much higher in those with meningo-encephalitis not related to mumps than in those with mumps meningo-encephalitis. Finally, symptoms were as a rule more severe in the adults than in the children.

The CSF findings in the different groups are given in Table 25. The examinations forming the basis of the data given in the table

Table 24. *Classification of material according to severity of symptoms 2 weeks after onset. (Figures in brackets indicate per cent.)*

	A	B	C	Total
Children:				
mumps meningo-enc.	30 (91)	3 (9)	0 (0)	33
other types of meningo-enc.	16 (59)	9 (33)	2 (7)	27
Adults:				
mumps meningo-enc.	7 (47)	6 (40)	2 (13)	15
other types of meningo-enc.	11 (25)	19 (43)	14 (32)	44
Total	64 (54)	37 (31)	18 (15)	119

A. Patients without symptoms.
B. Patients with moderate symptoms.
C. Patients with severe symptoms.

were with but few exceptions carried out during the third and fourth weeks of the disease. Only 11 observations – 4 belonging to group A, 4 to group B and 3 to group C – were made later during the course of the disease.

The number of cells in the CSF from children and adults with mumps meningo-encephalitis and belonging to group B did not differ appreciably from what was found in group A. Group C consisted of only 2 patients and therefore no comparison was made between groups A and C. Among the children and adults with meningo-encephalitis not related to mumps, however, the number of cells in the CSF was on the average higher in group C than in group A. The difference between the means was 2.7×ED. The groups also differed distinctly regarding the distribution among the different classes of cell-counts (Fig. 13).

The total protein content of the CSF in mumps meningo-encephalitis was roughly the same in all 3 groups. In adults with meningo-encephalitis not related to mumps the total protein content was on the average higher in groups B and C than in group A, and the ranges of individual variations were wider. The difference between the mean values in groups B+C and group A was 15 mg/100 ml (2.5×ED).

Table 25. *Cell content, total protein, albumin and gamma-globulin in cerebrospinal fluid 2 weeks or more after onset of acute abacterial meningo-encephalitis of varying severity.*

		Children						Adults					
		mumps meningo-enc.			other types of meningo-enc.			mumps meningo-enc.			other types of meningo-enc.		
		A	B	C	A	B	C	A	B	C	A	B	C
No. of cells per cmm	n	25	2	0	14	9	2	5	4	2	7	18	9
	m	9	14	—	16	13	48	8	13	31	14	27	42
	s	9	—	—	24	9	—	5	10	—	13	21	36
Total protein in mg per 100 ml	n	24	2	0	13	8	2	4	3	1	7	16	8
	m	24	39	—	23	26	83	39	36	36	42	58	55
	s	7	—	—	9	11	—	10	—	—	11	21	23
Albumin in per cent of total protein	n	24	2	0	14	9	2	5	3	2	7	18	9
	m	66.9	69.2	—	67.6	67.3	68.2	68.3	71.3	65.7	73.5	71.9	67.1
	s	6.4	—	—	6.4	5.7	—	4.3	—	—	4.9	5.4	8.7
Gamma-globulin in per cent of total protein	n	24	2	0	14	9	2	5	3	2	7	18	9
	m	7.2	7.0	—	7.3	8.4	12.9	8.1	7.2	12.1	7.1	8.7	11.4
	s	1.9	—	—	1.8	1.9	—	3.1	—	—	1.9	2.8	4.7

A. Patients without symptoms 2 weeks after onset.
B. Patients with moderate symptoms 2 weeks after onset.
C. Patients with severe symptoms 2 weeks after onset.

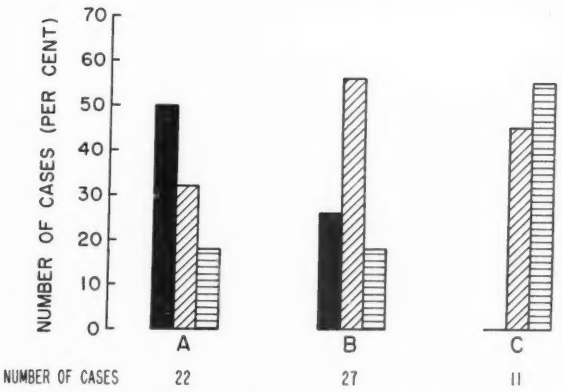


Fig. 13. Cell content in CSF more than 2 weeks after onset of acute abacterial meningo-encephalitis not related to mumps of varying severity. Distribution within different classes of cell-counts.

A. Cases without symptoms 2 weeks after onset of disease.
B. Cases with moderate symptoms 2 weeks after onset of disease.
C. Cases with severe symptoms 2 weeks after onset of disease.

Cases with <10 cells/cmm
Cases with 10—30 cells/cmm
Cases with >30 cells/cmm

Of the electrophoretically separated protein fractions of the CSF, differences were found between groups A, B and C only regarding the relative concentration of albumin and gamma-globulin. The other protein fractions were of roughly the same relative concentration in the different groups and are not accounted for here.

The relative concentration of albumin in the CSF of adults with meningo-encephalitis was occasionally lower in group C than in groups A and B. However, owing to the individual variations within the groups the differences between the means of the groups were too small to be statistically significant.

The relative concentration of gamma-globulin in the CSF was much higher in group C than in groups A and B. Among children and adults with meningo-encephalitis not related to mumps a cer-

B. Patients with moderate symptoms 2 weeks after onset.
C. Patients with severe symptoms 2 weeks after onset.

tain difference was found between the mean values of groups A and B. This difference was 2.2×ED and thus not quite significant. Between groups A and C the difference between the means was 3.2×ED and thus statistically significant. The range of individual variations was also much wider for group C than for the other two groups.

It was not possible to compare the groups with respect to the duration of the changes in the CSF because the fluid was usually not repeatedly examined until it had become normal. In those cases where the CSF was studied fairly long, however, the changes were sometimes found to persist for more than 3 months. Only 4 patients with pathologic CSF changes persisting for more than 3 months were seen in the present material — 2 children and 2 adults with meningo-encephalitis not related to mumps. Three of them belonged to group C and 1 to group B.

DISCUSSION

No certain relationship was found between CSF changes and severity of the symptoms at the time of onset of the disease in the patients with mumps meningo-encephalitis. On the other hand, in the patients with meningo-encephalitis not related to mumps, and especially in adults, a clear correlation was found between clinical symptoms and cellular and colloidal chemical changes in the CSF at onset of the disease.

No satisfactory explanation can be offered for this difference between mumps meningo-encephalitis and meningo-encephalitis not related to mumps. It might possibly be due in part to the heterogeneity of the causal factors in the latter group. One might thus imagine that the major part of group I happened to consist of certain etiologically defined forms characterised by both mild clinical symptoms and CSF changes and that group II was dominated by other etiologic forms of the disease with more pronounced clinical symptoms and CSF changes.

During later stages mumps meningo-encephalitis was much milder and the CSF returned to normal much earlier than in other types of meningo-encephalitis. It is therefore probable that the changes in the central nervous system in mumps meningo-encephalitis were relatively benign and that they were in the main localised to the meninges. Experience has also shown that the prognosis of the disease is good, and the fatal cases on record are very few (Bruyn, Sexton & Brainerd 1957).

In meningo-encephalitis not related to mumps, however, persistent symptoms were much more frequent and to a large extent accompanied by pronounced changes in the CSF. The cell content, total protein content and the relative concentration of gamma-globulin of the CSF was thus higher in patients who had severe symptoms than in the others.

These differences in the clinical picture and CSF findings among patients with meningo-encephalitis not related to mumps might to a certain extent be explained by the etiologic heterogeneity of the material. Besides there is probably a variation in the individual mode of reaction to the causal agents. In the present material persistent symptoms and pronunced changes in the CSF were thus more frequent among adults than among children.

It is difficult to judge to what extent the brain was involved by the pathologic process in the present material. Symptoms of encephalitis in the form of persistent pareses or tendency to convulsions were noted in only 2 cases. It is true that the symptoms dominating the picture during the later course suggested cerebral involvement, above all in the group with the most severe symptoms, but they may also have been partly of meningeal origin.

Electro-encephalography was performed in 58 cases (13 with mumps meningo-encephalitis and 45 with meningo-encephalitis of other origin). Of the recordings, 17 showed slight abnormalities in the form of mild theta dysrhythmia, while 9 showed more pronounced pathologic changes with delta focus. The remaining 32 recordings were of normal appearance. The frequency of abnormal

recordings was the same in all 3 groups, though pronounced pathologic changes with delta focus were more common in group C (36 per cent of all recordings as against 7 per cent in group A and 12 per cent in group B). In none of these groups did the patients with electro-encephalographic abnormalities differ regarding the CSF findings from those with normal electro-encephalograms.

Of particular interest was the increase in the relative gamma-globulin concentration in patients with severe symptoms. In those cases in which the increase was relatively moderate it was probably ascribable to differences in reabsorption of the protein fractions from the CSF space to the blood stream.

In other cases in which the gamma-globulin increase was more marked and persistent, however, one might imagine a cerebral or meningeal site of production. The increase in the gamma-globulin of the CSF would then be comparable to the gamma-globulin increase occurring in neurosyphilis and multiple sclerosis, where it is believed to be due to formation of antibodies in the central nervous system. However, the present material included only 2 cases with a marked — over 20 per cent of the total protein — and persistent gamma-globulin increase: therefore no valid conclusion can be made on this point.

SUMMARY

While no relationship was found between the CSF changes and the clinical picture during the acute stages of mumps meningo-encephalitis, a clear correlation was demonstrable in meningo-encephalitis of other origin between the clinical symptoms at onset and the cell and protein content of the CSF.

During the later course of the disease most patients with mumps meningo-encephalitis were symptom-free. In meningo-encephalitis not related to mumps, however, persistent symptoms in the form of tiredness, headache, dizziness, loss of memory, concentration difficulties, irritability, abnormal intolerance of light and sound were much more frequent and to a large extent accompanied by pro-

nounced changes in the CSF: marked increase in the cell content, protein content and the relative concentration of gamma-globulin.

Judging from the results obtained, the changes in the central nervous system in mumps meningo-encephalitis are probably relatively benign and localised mainly to the meninges. The other meningo-encephalitides represented a heterogenous group with respect to the etiology, severity and duration of the disease and probably also regarding its pathologic-anatomic localisation.

In addition to the etiology of the disease, the clinical picture was probably dependent on variations in the individual mode of reaction. Thus, in the material examined an increase was noted in the frequency of clinical symptoms and pathologic CSF findings with age.

The increase in the relative concentration of gamma-globulin in patients with severe symptoms might be ascribable in part to a relative retardation of the reabsorption to the blood stream. However, in cases with pronunced increase of the gamma-globulin the possibility of a cerebral or meningeal site of production must be considered.

No correlation was found between the electro-encephalographic findings and the changes of the CSF in the material.

GENERAL SUMMARY

In an investigation of the cell content, total protein content and the electrophoretic protein pattern of 123 CSF samples from 98 normal children, aged 0–13 years, the following observations were made.

The cell content of the CSF was higher during the first 3 months of life than later in childhood. The range of variation of the individual values was considerable, above all during the first weeks of life.

The total protein content of the CSF was also larger during the neonatal period than during the rest of childhood. During the first year of life the mean protein content decreased successively, reached a minimum between the ages of 9 months and 2 years, then began to increase again and continued to do so throughout the rest of childhood.

Of the electrophoretically separated protein fractions of the CSF, albumin in its variation with age showed a distinct parallelism with the total protein content. The mean albumin concentration in the CSF – expressed in per cent of total protein – was thus highest during the neonatal period and lowest in children 9 months to 2 years of age, after which it again succesively increased. The other protein fractions of the CSF, except gamma-globulin, showed roughly the converse variation.

During the first week of life the relative concentration of albumin in the CSF was higher than in the serum. The physiologic basis of this is presumably mechanical irritation of the meninges in association with delivery and subsequent increase of passage of serum protein into the CSF space, especially of the relatively small dispersed albumin molecules.

The variations in the amount and composition of the CSF protein in different ages probably depend on variations in the relative amount of the admixture of serum protein during passage of the CSF from the ventricles to the lumbar subarachnoidal space. The increased addition of serum below 9 months of age is probably due to an increased permeability of the blood-CSF barrier. The changes occurring after 2 years are probably related to a relatively decreasing function of the choroidal plexus with increasing age.

Investigation of CSF from 31 adults showed a continued increase in the total protein content and the relative albumin concentration of the CSF as well as a continued decrease in the relative concentrations of beta-globulin and X-fraction. These observations suggest a further relative decrease in the CSF production by the choroidal plexus.

Analyses of 256 CSF samples from 119 patients, including 60 children, with acute abacterial meningo-encephalitis revealed as follows.

At the time of onset of the disease the cell content of the CSF was usually higher in mumps meningo-encephalitis than in meningo-encephalitis of other origin. While no relationship was found between the CSF changes and the clinical picture during the acute stages of mumps meningo-encephalitis, a clear correlation was demonstrable in meningo-encephalitis of other origin between the clinical symptoms at onset and the cell and protein content of the CSF.

During the later course of the disease most patients with mumps meningo-encephalitis were symptom-free. In meningo-encephalitis not related to mumps, however, persistent symptoms in the form of tiredness, headache, dizziness, loss of memory, concentration difficulties, irritability, abnormal intolerance of light and sound were more frequent and to a large extent accompanied by pronounced changes in the CSF: marked increase in the cell content, protein content and the relative concentration of gamma-globulin.

Of the electrophoretically separated protein fractions of the CSF,

the albumin during the initial stage of the disease was markedly increased, the relative concentration exceeding that in serum, at the same time as the other protein fractions of the CSF were below normal. During the further course the pattern gradually became normal although the relative gamma-globulin concentration was above normal for some time afterwards, especially in patients with severe symptoms. The increase in the gamma-globulin concentration occasionally persisted for more than 3 months.

Judging from the results obtained, the changes in the central nervous system in mumps meningo-encephalitis are probably relatively benign and localised mainly to the meninges. The other meningo-encephalitides represented a heterogenous group with respect to the etiology, severity and duration of the disease and probably also regarding its pathologic-anatomic localisation.

In addition to the etiology of the disease, the clinical picture was probably dependent on variations in the individual mode of reaction. Thus, in the material examined an increase was noted in the frequency of clinical symptoms and pathologic CSF findings with age.

The abnormalities in the electrophoretic pattern of the CSF protein are probably due to disturbed meningeal permeability. During the initial stage of the disease serum albumin will pass into the CSF space more readily than the remaining serum protein fractions. The increase in the relative concentration of gamma-globulin during the later course of the disease might partly be ascribable to a relative retardation of the reabsorption to the blood stream. However, in cases with a pronounced increase of the gamma-globulin the possibility of a cerebral or meningeal site of production must be considered.

No correlation was found between the electro-encephalographic findings and the changes of the CSF in the material.

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